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Using multimodal MRI and real-time fMRI neurofeedback to understand the mechanism of attentional control in people with high trait-anxiety

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Using multimodal MRI and real-time fMRI neurofeedback to understand the mechanism of attentional control in people with high trait-anxiety

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For from him and through him and to him are all things.

To him be glory for ever.

Amen.

Romans 11:36

Frequently used Abbreviations and Acronyms

^{1}H-MRS	Proton Magnetic Resonance Spectroscopy
ACC	Anterior Cingulate Cortex
ACT	Attentional Control Theory
CON	Cingulo-Opercular Network
CPT	Continuous Performance Task
DASS	Depression Anxiety Stress Scale
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
EPT	Emotional Probe Task
fMRI	functional Magnetic Resonance Imaging
FPN	Fronto Parietal Network
GABA	gamma-Aminobutric acid
Glu	Glutamate
HTA	High Trait Anxiety
ICA	Independent Component Analysis
LTA	Low Trait Anxiety
NMDAR	N-methyl-D-aspartate receptors
PCC	Posterior Cingulate Cortex
PFC	Prefrontal Cortex
PPI	Psychophysiological Interaction
RSFC	Resting-State Functional Connectivity
Rt-fMRI-nf	Real-time fMRI-neurofeedback
STAI	State Trait Anxiety Inventory
VAN	Ventral Attention Network

Abstract

Attentional Control Theory is a framework describing how High Trait Anxiety (HTA) impairs performance during attentional control tasks. In this thesis empirical studies were performed to investigate how HTA affects the neural substrates of attentional control *and* if real-time functional Magnetic Resonance Imaging neurofeedback (rt-fMRI-nf) could be used to improve attentional control and reduce anxiety in HTA individuals.

First, in a combined fMRI- ^{1}H-Magnetic Resonance Spectroscopy study, a Stroop task was used to elicit functional activation in Dorsolateral Prefrontal Cortex (DLPFC) and Anterior Cingulate Cortex (ACC). Prefrontal Cortex (PFC) Glutamate (Glu) levels were also measured in the same individuals. HTA participants showed reduced task performance relative to Low Trait Anxiety (LTA) participants. Furthermore, there was a positive association between PFC Glu and DLPFC activation during incongruent trials in LTA participants but not in HTA participants, indicating a possible mechanism for impaired attentional control in HTA individuals.

The second series of studies examined the feasibility of rt-fMRI-nf for enhancing DLPCF–ACC functional connectivity and activity in HTA individuals. Trait anxious participants were assigned to either an experimental group, undergoing veridical rt-fMRI-nf, or a control group, receiving sham feedback. Post-rt-fMRI-nf, the experimental group (EG) showed reduced anxiety levels and increased DLPFC-ACC functional activity and connectivity relative to the control group (CG). Resting State Functional Connectivity (RSFC) and attentional control performance were also assessed pre- and post-rt-fMRI-nf. Whilst connectivity-based rt-fMRI-nf increased RSFC in the Posterior Cingulate Gyrus, there were no effects of rt-fMRI-nf on offline task performance.

It was shown that trait anxiety affects the relationship between PFC Glu and DLPFC activation, possibly contributing to ineffective task performance when attentional control is required. Furthermore, DLPFC-ACC functional connectivity-based rt-fMRI-nf, led to reduced anxiety and changes in neural activity that could be interpreted as increased *processing efficiency* in brain circuitry, important for attentional control. However, there were no measurable improvements in task performance.

Table of Contents

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1. General Introduction and Literature Review

1.1. Attentional Control and Anxiety

Anxiety disorders characterised by excess worry, hyperarousal, and debilitating fear are some of the most common psychiatric conditions in the world with an estimated lifetime prevalence of 14.3% [1]. Moreover, trait anxiety is part of the 'normally distributed' personality dimension of neuroticism that is characterised by intrusive thoughts, worry and difficulty in disengaging from negative material [2]. Importantly, high trait anxiety (HTA) is believed to be a general predictor and risk factor for anxiety disorders [3].

Trait anxiety as a relatively stable personality trait and general proneness to anxiety has been distinguished from State anxiety, the temporary state of intense apprehension and worry in response to particular environmental events or stressors [4]. Psychologically, trait anxiety manifests in worry (i.e. anxious apprehension), a construct understood as the cognitive component of trait anxiety, however, individuals with HTA also experience higher levels of physiological arousal [4].

Over recent decades much research has been undertaken to examine how trait anxiety affects cognitive performance [5-7]. This is because trait anxiety and worry have a strong cognitive component; i.e. people with HTA often have difficulties concentrating; as attentional control can be compromised by a bias towards negative or threat-laden information [8] or by worry competing for limited cognitive resources [9]. This is thought to

be a fundamental bias seen in anxiety disorders that contributes to *and* maintains symptomology [10].

Psychological models of attention [7, 11] posit that the cognitive ability to inhibit irrelevant information is a key component of effective attentional control [12]. In people with anxiety, an impaired ability to inhibit negative or threatening stimuli or direct attention away from these stimuli may contribute to attentional control problems, whilst enhancing bias to negative stimuli [7, 12]. However, more general impairments in attentional control, independent of threat-related information, have been reported [6, 13-15]. Different explanations have been offered to account for these impairments, such as increased cognitive load due to increased distractibility, cognitive resources allocated to worry and anxiety (see [6, 16]) or inefficient cognitive processing as a result of anxiety (see [7, 13]).

1.1.1. *Theoretical Framework of Attentional Control Theory*

Attentional Control Theory (ACT, [7]) has been a highly influential model that provides a framework for understanding how anxiety and trait anxiety can affect cognitive processes and attentional control. Importantly, with its many theoretical assumptions, ACT has proved a useful framework for empirical work (See [17] for review). The theory has its origins in an earlier conceptualization by Eysenck [18] known as Processing Efficiency Theory (PET; [19]), and has since been developed into ACT [7] which has in turn been further developed in light of new empirical research [20, 21].

Central to ACT, is the prediction that trait anxiety impairs *processing efficiency* (the quality of performance relative to use of processing

resources) more than *performance effectiveness* (quality of performance, usually measured by error rates (ERs) and reaction times (RTs)). Moreover, the framework attributes efficient cognitive processing to the functioning of the central executive (an attention-like system of major importance within Baddeley's working memory model [22-24] localised in the Prefrontal Cortex (PFC); [25]).

The central executive is a system of limited capacity that regulates attentional resources. Miyake and colleagues [26] determined three major function: *inhibition* (the ability to inhibit irrelevant or distracting information), *shifting* (the ability to direct attention to task relevant information) and *updating* (the ability update task directed goals when the requirements of a given task change). ACT makes specific predictions that in people with HTA *processing efficiency* is impaired when inhibition or shifting are required. In contrast, tasks requiring updating are only impaired under stressful conditions, as updating itself does not impose as strong demands on attentional control as inhibition and shifting. The behavioural evidence for impairments of the inhibition and shifting function have been outlined in much detail in the original publication of ACT and subsequent publications (see [7, 20, 21]).

The framework further postulates that highly trait anxious individuals often utilise compensatory strategies to overcome the inefficiency caused by distracting exogenous and/or endogenous stimuli such as distractors or other non-task processing, such as worry. These compensatory processes allow trait anxious individuals to achieve comparable performance to non-

anxious individuals. Hence *performance effectiveness* is retained, albeit using less efficient mechanisms i.e. using greater cognitive and/or neural resource without a concomitant improvement in *performance effectiveness*. Consequently, adverse effects of HTA on performance occur more frequently when central executive demands are high, due to the limited nature of executive control resources and the demand of compensatory strategies on them.

According to ACT, trait anxiety disrupts the balance between goal-directed attentional system and stimulus-driven attentional system, while the influence of the latter is increased. The goal-directed attentional system is directed by knowledge and current aims, while the stimulus-driven attentional system is influenced by salient and conspicuous stimuli. The heightened influence of the stimulus-driven attentional system is particularly evident in the threat-related biases frequently observed in people with HTA. Threat-related bias is seen towards both external and internal threat-related stimuli, although bias towards internal stimuli in the form of worry is more difficult to measure. Consequently, there is limited empirical evidence for its effects [7].

Other theoretical approaches on the relationship between attentional control and anxiety share the notion of impoverished attentional control in people with high anxiety, however, such approaches differ in the predictions of the specific processes involved in impairments of attentional control. For instance Bishop [6], while their results are partly consistent with ACT, attribute impaired attentional control in people with high

anxiety to impoverished recruitment of attentional control resources in the PFC.

1.1.2. *Attentional Control and Anxiety in Psychological Research*

Eysenck and colleagues [7, 20, 21] provide a detailed account of the empirical evidence for ACT from behavioural studies. There is a large body of behavioural evidence supporting the claim that anxiety consistently impairs the efficiency of the inhibition and shifting functions [17, 27]. Performance effectiveness is easily measured with different outcomes in behavioural tasks (e.g. ERs). Investigating processing efficiency with behavioural methods is more complex. Nevertheless, there has been some work to quantify impairments in processing efficiency. One method is using task conditions with increasing load on attentional control functions. A stereotypical result is that compared to control, people with high anxiety show comparable RTs in low load conditions, but slower RTs when task demands are high compared to control (e.g. [28], testing the shifting function). An interaction between load and anxiety indicates inefficient processing. Another way of measuring processing efficiency is using tasks that follow saccadic eye movements, for instance the antisaccade task (e.g. [17, 29, 30]). Berggren and Derakshan [17] show that while high anxiety was not associated with increased ERs when demands on the inhibition function are high, inhibitory cost (as measured with saccade latencies) was positively correlated with anxiety.

Behavioural studies also confirm that high-anxious individuals are more susceptible to distraction than low-anxious ones because their stimulus-driven attentional system is generally more active [17, 31-33].

However, the main focus of this thesis is the understanding of underlying neural mechanisms of attentional control. It concerns itself with how these mechanisms relate to established functional networks in the brain, and how these mechanisms and networks are affected by trait anxiety.

1.1.3. *Attentional Control and Anxiety in Neuroimaging Research*

Eysenck and colleagues [7] predict inefficient processing in people with HTA, which are expressed in neural inefficiency of relevant processes in the brain (i.e. the quality of performance relative to use of neural resources). However, they make only a few predictions about how trait anxiety impacts specific neural processes in relationship to cognitive control. Hence, while ACT is the underlying theoretical framework to this research, the precise predictions at a neurofunctional level are also based on independent neuroimaging work that investigates how ACT translates to brain function (e.g., [13, 15, 34]). In this section, empirical work from the field of cognitive neuroscience that relates to ACT will be reviewed and it is outlined so to show how the predictions of ACT may translate to a neurofunctional level.

There has been a shift in cognitive neuroscience from considering separate brain regions and their activation and deactivation to evaluating brain function based on interconnected functional and resting-state networks. This is because numerous Magnetic Resonance Imaging (fMRI) studies

indicate that certain processes are consistently associated with widespread activation within several brain areas [35]. Consequently, the following will focus on specific networks and regions that have been implicated in attentional control, and examine how these networks and regions are affected by trait anxiety.

Attentional control has been linked to a number of brain networks and regions. The fronto-parietal network (FPN), the ventral-attention network (VAN), the cingulo-opercular network (CON) and the default mode network (DMN) are all believed to play a role in effective and efficient attentional control [36]. Furthermore, there is a body of emerging evidence from fMRI studies that anxiety can affect the functioning of, and interactions between, these networks.

The FPN includes the Intraparietal Cortex and Superior Frontal Cortex including the Inferior Parietal Lobule, Middle Cingulate Cortex and Precuneus. These regions are important for attentional control [36, 37] and are attributed to the goal-directed or 'top-down' attentional system [7, 37-39]. The stimulus-driven attentional system is associated with the VAN, including the Temporo-Parietal Junction and ventral PFC, and may also depend on activation of CON [36] (also 'salience network' [40]) encompassing the Anterior Cingulate Cortex (ACC) and bilateral insulae. The function of the CON entails error-monitoring activities, which are important for reactive attentional control. Lastly, activity in the DMN, encompassing the Posterior Cingulate Cortex (PCC), precuneus, medial PFC and lateral Parietal Cortex, is known to be altered in individuals with

HTA [36, 41]. The DMN is a task negative network (i.e. it is deactivated during task processing and activated during rest). The DMN is anticorrelated to a task positive network, described as extrinsic mode network (EMN) by Hughdal and colleagues [42], this includes FPN, VAN and CON. The DMN is important for task-irrelevant thinking including mind-wandering [43] and attentional lapses [44], it has also been associated with emotion regulation [36].

It is believed that the interaction between the FPN and VAN, representing goal-directed and stimulus-driven attentional systems respectively, is crucial for effective attentional control [45]. Furthermore, the CON has an important role for reactive attentional control, needed to update the FPN when distractors are present or task demands change. However, along with the DMN, it is thought that all four networks interact during attentional control to achieve efficient neural processing, a process that is thought to be dysfunctional in highly trait anxious individuals [36].

Other regions in the brain are also associated with either anxiety or attentional control. However, for the purpose of this review, the focus will remain on those regions and networks believed to be important for attentional control processes and thought to be impaired or altered in HTA individuals.

1.1.3.1.Evidence from Electroencephalogram Studies

Several Electroencephalogram (EEG) studies have investigated the relationship between attentional control and trait anxiety. With its superior temporal resolution to fMRI, EEG lends itself to the investigations of

temporal patterns of brain activation associated with attentional control and/or attentional bias to threat stimuli. The majority of EEG research on attentional control and anxiety focuses on error monitoring localised in the CON. However, a small number of EEG studies have also provided some insight into dysfunctional interactions between networks, in particular CON and FPN.

A well-studied substrate of anxiety in EEG research is increased amplitude of the error-related negativity (ERN), an event-related potential (ERP) reflecting error-monitoring functions believed to be generated in the dorsal ACC (dACC) within the CON. ERN typically occurs 100 ms after an erroneous response in RT tasks [46]. Recent research by Hoffman and colleagues [47] suggests that the ERN reflects processing related to conscious errors in trials with high uncertainty, rather than reaction slips. They employed a combined EEG and fMRI design and a mental rotation task in healthy participants, demonstrating that ERN is reflective of a cognitive process of inhibiting errors and replacing them with the correct responses. Thus, enhanced ERN in HTA individuals is consistent with the predictions of ACT; that anxiety reduces the efficiency of the inhibition function of executive control and a greater compensatory ERN is needed for successful inhibition of task irrelevant stimuli. Importantly for the predictions of ACT, this pattern of enhanced ERN signal in HTA individuals is usually seen without a concomitant improvement in task performance [48]. Using the terminology of ACT, this is demonstrative of *processing inefficiency*, whilst *performance effectiveness* remains unchanged.

ERN has been consistently localised to the ACC and may reflect the activity of a coordinated network involving communication between ACC and Dorsolateral Prefrontal Cortex (DLPFC) engaged within 100 ms of an erroneous response [49]. Moser and colleagues [48] report a negative correlation between ERN amplitude during an Eriksen Flanker task and worry scores, indicating that stronger dACC reaction to errors may serve as a compensatory mechanism due to endogenous distraction through worry. Moreover, in a meta-analysis of 37 studies using standard conflict tasks (e.g., the Stroop task; the go/no-go task, the Eriksen Flanker task), Moser and colleagues. [46] found anxiety was associated with a larger ERN. They concluded, 'Enhanced ERN in anxiety may index a compensatory effort signal aimed at maintaining a standard level of performance'. In this meta-analysis a wide range of non-clinical and clinical types of anxiety were considered. Of theoretical relevance, and consistent with the prediction of ACT, anxiety typically had no effect on performance, suggesting the enhanced ERN found in high-anxious individuals may be part of a process to maintain performance levels.

Related to ERN is correct-response negativity (CRN), another frontal EEG component that reflects response conflict during attentional control. There was a significant interaction between CRN amplitude and social trait anxiety on ERs in a response conflict task (a version of the Eriksen Flanker task) [50]. In particular, smaller CRN amplitude predicted worse response control in people with high social trait anxiety. In the same study, there was no effect of social trait anxiety on PFC EEG asymmetry (measure of proactive/top-down control in the DLPFC). These results indicate a greater

compensatory reliance on conflict monitoring mechanisms in people with high social trait anxiety that is absent in people with low social trait anxiety. Notably, Schmid and colleagues [50] insist on a theoretical differentiation of social trait anxiety from general trait anxiety; they claim that their results are specific to social anxiety, while they did not directly test this.

More ERP-patterns have been associated with increased amplitudes in high anxiety such as the N2 component, a component reflecting cognitive control processes in the Frontal Cortex [51] during a go/no-go inhibition task. These enhanced N2 responses most likely reflect inhibitory attentional control processes occurring within the Frontal Cortex, while some research attributes a more general role of conflict monitoring to N2 [52]. Since there were no effects of trait anxiety on performance (RTs and accuracy) in this study, the overall findings suggest that, consistent with the predictions of ACT, anxiety impaired *processing efficiency* rather than *performance effectiveness*.

EEG research also shows significant differences between low and HTA individuals in alpha and beta frequency desynchronisation, before and after a button press during an inhibition task (stop-signal paradigm; [53]). High anxious participants showed stronger power decrease during task processing. This can be explained with higher alpha and beta power during resting, reflecting compensatory efforts in preparation for the task. Savostyanov and colleagues [53] argue, in line with ACT, that people with HTA employ more resources towards attentional control.

With regards to attentional bias to threat, a feature of impaired attentional control in HTA, as described by ACT [7], Fisher and colleagues [54] demonstrated in an emotional Stroop task that people with HTA display faster ERPs in response to negative stimuli in frontal and parietal regions (FPN). Similar results were reported using an emotional probe task (EPT; [55]). People with HTA exhibited increased neural reactivity to angry faces compared to people with Low Trait Anxiety (LTA). These studies indicate increased early activity in a bottom-up attentional system specifically related to threat in people with HTA.

Lastly, consistent with ACT, several EEG studies indicate network inefficiencies in people with HTA. Putman [56] reports that selective attention to threat in a dot-probe task is associated with altered resting-state EEG frequency band power in frontal regions. Putman uses δ–β coherence as a measure of functional synchronisation between limbic and cortical systems in the brain. In the study, greater attention to threat was related to desynchronization of δ–β coherence. Furthermore, there was stronger δ–β coherence in people with LTA compared to people with HTA. This indicates reduced connectivity between limbic and cortical function in people with HTA is related to performance during an attentional control task, and that anxiety is associated with inefficient communication between regions involved in bottom-up and top-down attentional processing. In a later study Putman and colleagues [57] did not replicate their earlier finding linking trait anxiety and altered δ–β coupling. However, there was a significant association between attentional bias and reduced δ–β coupling in frontal regions, which may show that efficient

connectivity between top-down and bottom up attentional systems is needed for optimal attentional control. Using advanced EEG signal processing analysis [58], Moran and colleagues [27] found worry was associated with reduced coupling between dACC and lateral DLPFC recording sites on error trials during an Eriksen Flanker task. ERP findings also provide support for the prediction that during attentional control, people with high levels of trait anxiety employ compensatory neural mechanisms to maintain effective performance. Using path analysis to model the effects of anxiety on ERN and ACC-DLPFC coupling, Moran and colleagues [27] demonstrated that the enhanced ERN in anxious individuals compensated for reduced ACC-DLPFC coupling, thereby stabilizing post-error performance relative to lower anxious individuals.

1.1.3.2.Evidence from functional Magnetic Resonance Imaging

FMRI indirectly measures brain activation via changes in oxygenated cerebral blood flow. Following neural activation the respective brain regions are supplied with increased levels of oxygenated blood in a process of homeostasis. The peak concentration of oxygenated blood after neural activity is typically reached after 4-6 seconds, constituting a natural delay in the fMRI signal. Deoxygenated and oxygenated blood display different magnetic properties, thus the MRI scanner can measure the hemodynamic response of the brain. Notably, fMRI is an indirect measure of neural activation; and its temporal resolution is considerably slower than the pace of neural firing [59, 60].

Several decades of fMRI work has localised the goal-directed attentional system to the FPN centred in the PFC [7, 11, 61]. ACT predicts altered brain activation in the FPN, in particular the bilateral DLPFC, as well as more general network inefficiencies related to top-down attentional control [7]. Much of the available evidence points towards high-anxious individuals often having greater DLPFC activation than low-anxious individuals during tasks that require executive functions (e.g., inhibition; shifting; updating). This increased activation is interpreted as compensatory effort in an inefficient attentional control system and is expected to be greater when demands are high on attentional control. Basten and colleagues [13] assessed right DLPFC activity during a colour-word Stroop task. HTA participants showed a significantly greater increase in DLPFC activation than LTA individuals during incongruent trials only, which require greater cognitive resources. Since the effects of trait anxiety on performance were non-significant, the effects of anxiety seemed to affect *processing efficiency* rather than *performance effectiveness*, again consistent with the predictions of ACT.

It is an important observation that during congruent Stroop trials (low cognitive load) no effect of trait anxiety were observed, as this supports the prediction of ACT that increased DLPFC activation is compensatory, *and* is dependent on task requirements (i.e. compensatory efforts to maintain *performance effectiveness* are only required when task demands are high). Similar findings have been reported in relation to working memory function [14]. Basten and colleagues [14] report that there was a greater increase in task-related activation in DLPFC in HTA individuals

compared to LTA individuals during a working memory manipulation condition (requiring the updating function) than in a maintenance condition that did not require the updating function. Again, these findings suggest that DLPFC activity is greater in anxious individuals when executive function demands are greater, thus reflecting compensatory efforts.

Fales and colleagues [15] also used a working-memory task requiring the updating function (the n-back task). Their most relevant finding was that high-anxious individuals showed reduced sustained activation in the FPN (possibly reflecting engagement of the FPN during proactive control) but increased transient FPN activation, during a working memory task, reflecting the use of compensatory processes. Again, anxiety had no effect on task performance and Fales and colleagues [15] speculate, consistent with ACT, that the balance between top-down and bottom-up attentional system is altered in people with high anxiety.

However, not all fMRI findings are consistent with ACT. Bishop [6] used two versions of an fMRI letter search task requiring inhibition of a distractor. While one task involved minimal perceptual load, a second harder task involved much greater perceptual loading, this was achieved by manipulating the complexity of the distractor stimulus used. It was reported that anxiety was associated with greater DLPFC activation in the more difficult task condition. This is consistent with the predictions of ACT, though Bishop [6] did not acknowledge this in the discussion of her findings. In the task with minimal perceptual load, trait anxiety was linked

to impoverished/reduced recruitment of PFC activation during the inhibition of distractors, while there were no group differences in task performance. During the low load task Bishop [6] reported a strong negative correlation between trait anxiety and DLPFC activation, a finding seemingly inconsistent with ACT. However, within the framework of ACT, increased DLPFC activation is only predicted, when executive functions are required (i.e. to compensate for reduced *processing efficiency*). In a condition not requiring increased neural effort to maintain *performance effectiveness* it is very possible, that reduced DLPFC activation was observed, reflecting reduced functioning of the goal-directed attentional system.

Another study using an emotional interference task [62] also reports a negative relationship between trait anxiety and DLPFC activation. These findings are seemingly in conflict with other research (e.g., [13]) and the theoretical framework of ACT. ACT would predict that the conflict between task stimuli and distractors should produce compensatory processing activity (including within DLPFC). Such a pattern was observed in the high load task used by Bishop [6], but the opposite pattern was seen during the low perceptual load condition. It is possible that during the low load condition compensatory processing was not required, although this is not fully consistent with the pattern of reduced DLPFC activity in anxious individuals. The reduced activation in the DLPFC, therefore, would reflect reduced functioning of the FPN in task preparation (see dual mechanisms of control theory [63]). This finding is not wholly inconsistent with the predictions of ACT; however, the model makes

limited predictions regarding neural activation during rest or low load conditions.

Similarly, Forster and colleagues [64] report, using a go/no-go task, that during less frequent no-go trials, anxiety was associated with reduced sustained DLPFC and ACC activation and increased transient activation in attentional control regions. These results reconcile previous inconsistent findings questioning whether attentional control regions are more or less activated in people with HTA, by distinguishing sustained and transient activation. Less activation during low load conditions may reflect impoverished recruitment of attentional control networks, while increased activation during task processing may be due to increased compensatory effort. Another study using a similar go/no-go paradigm [65] also used fMRI to compare brain activation in high and low-anxious individuals. Trait anxiety did not affect performance. However, when the task required inhibitory control (i.e. on no-go trials), high anxious individuals had greater activity than low-anxious ones in DLPFC and temporo-parietal brain regions, a finding consistent with ACT.

Whilst the fMRI research discussed above has contributed to the understanding of how trait affects brain regions and networks involved in attentional control, little research has been conducted to better understand the temporal dynamics of these processes and how they are affected by anxiety. Silton and colleagues [66] provide some insight into the temporal mechanism of ACC and DLPFC interactions in healthy participants combining both fMRI and EEG during a colour-word Stroop task. It was

shown that, during attentional control, dACC activity followed DLPFC activity. The temporal course of these findings suggests dACC activity works as a compensatory mechanism during attentional control, presumably when DLPFC mediated top-down control is inefficient and/or ineffective.

Although less researched than the goal-directed attentional system, the stimulus-driven attentional system or ventral attentional network (VAN) is associated with the temporo-parietal and ventral PFC activation [11] and may also depend on activation of the 'salience network' (CON [36]) encompassing the ACC and bilateral insula. Both these systems have been shown to have increased functioning in highly anxious individuals, resulting in an overactive stimulus-driven attentional system and increased sensitivity to errors [36]. The notion of attentional bias in people with HTA is widely accepted to be due to an overly reactive 'bottom-up' attentional system that is sensitive to distracting stimuli. It is possible that the VAN is more active in high-anxious individuals as a consequence of over-vigilance to exogenous threat. This is consistent with reports of aberrant functional connectivity with Limbic regions, such as the Amygdala and Orbitofrontal Cortex, resulting in hypervigilance [67].

The effects of trait anxiety on the CON are mostly related to over-activation in the ACC and most likely best studied with methods that are not limited by the sluggish hemodynamic response associated with fMRI. Nevertheless, a number of fMRI studies have examined the effects of trait anxiety on ACC activity and their findings need to be considered. The

dorsal ACC (dACC) as the hub of CON is important for detecting conflict (i.e. a mismatch between pre potent and correct responses) and signalling the need for increased cognitive or attentional control to the DLPFC [68, 69]. According to ACT, because HTA individuals have less efficient top-down control (i.e. inefficient FPN functioning). They need to devote more resources to conflict detection and consequently exhibit greater dACC activation on conflict tasks [7].

Eisenberger, Lieberman and Satpute [70] used an oddball task in which a different response was required on infrequent oddball trials. The extent to which dACC activity was greater on oddball trials than non-oddball trials correlated positively with neuroticism, probably reflecting compensatory control in anxious individuals. This is consistent with other studies showing increased ACC activity associated with greater trait anxiety during task processing, while performance is not always affected [71]. In contrast, in generalised and social anxiety disorder cohorts, one study found decreased activation in the dACC and parietal regions during an emotional interference task compared to healthy controls [72]. This finding is inconsistent with research in trait anxiety samples, but consistent with other clinical work on generalised anxiety disorder showing decreased rACC activation [73]. These findings may be due to the very specific patient groups and tasks used. However, one study by Klumpp and colleagues [62] does describe a similar pattern of reduced activation in the rACC associated with greater trait anxiety during an emotional interference task.

Besides altered task-related activation, ACT also predicts increased processing of task-irrelevant internal stimuli (endogenous distraction). Brain-imaging research in the area of endogenous distraction and task-irrelevant processing is relatively limited. However, there is increasing evidence that worry and mind wandering both involve the DMN, and that high anxiety is associated with higher DMN activation [74]. First, the evidence on mind wandering was subjected to a meta-analysis [43]. Mind wandering and spontaneous thought were associated with activation in several key regions within the DMN (and other non-DMN regions). Second, a link between DMN activity and worry has been demonstrated [75] in a study that found that requesting participants to worry about a topic led to increased DMN activation. Gentili and colleagues [76] also further found that DMN activity was correlated positively with social anxiety scores and identified that higher levels of social anxiety were positively associated with DMN activity during task performance [77]. Importantly activity in the DMN is anti-correlated with activity in attentional control networks [35]. Consequently, increased DMN activation may disrupt anti-correlations between DMN and attentional control networks leading to impaired task performance [44, 78]. Pletzer and colleagues [78] found less deactivation in DMN regions during task performance for individuals high in math anxiety. However, in contrast to the majority of literature Fales and colleagues [15] detected increased deactivation of DMN in high-anxious individuals during a working memory task, a finding not consistent with the view stated above. Whilst the exact nature of the relationship between DMN activity and anxiety is unclear, a number of

studies suggests that anxiety is associated with dysfunction within the DMN. Task-related deactivation of DMN is also altered, but may depend to some extent on the type of task and its reliance upon executive processes [44].

In addition to the effects of anxiety on individual functional networks, it is also important to consider interactions between functional networks. Different networks in the brain have been found to be either correlated or anti-correlated and these network interactions are important for cognitive function. These network interactions are altered in HTA [35, 36]. Most relevant for attentional control, functional connectivity (i.e. the temporal correlation between structurally distinct brain regions) between FPN regions, specifically the DLPFC, and the ACC is reduced or altered in people with HTA [71]. As discussed earlier, the ACC is thought to be important for 'reactive' or 'compensatory' attentional controls [37] and updating the DLPFC when increased cognitive control is required [79, 80]. Consequently, efficient connectivity between these regions is thought to be important for maintaining effective and efficient attentional control [81]. Consistent with observed impairments in attentional control, people with high anxiety show reduced connectivity between DLPFC and ACC during tasks requiring attentional control [27, 82]. Using an emotional interference task, Comte and colleagues [71] showed that task-related functional connectivity between the ACC and lateral PFC is reduced in high-anxious relative to low-anxious individuals, suggesting disrupted coupling and communication. Similarly, Basten and colleagues [13] report that trait anxiety predicts functional connectivity between the DLPFC and

brain regions known to be involved in attentional control, including the ACC. DLPFC and ACC represent the FPN and CON respectively, both networks important for attentional control [83].

Taken together, brain-imaging studies using fMRI have reported that trait anxiety is associated with inefficient neural activity and altered connectivity during attentional control tasks [13-15, 65, 82]. Anxious individuals show greater activation in FPN and CON regions, when performing tasks requiring executive function and reduced functional connectivity, particularly between the DLPFC and ACC, both important hubs in the FPN and CON respectively. These findings are broadly in line with EEG findings of increased frontal signals in people with high degrees of worry when executive function is needed. Respectively, EEG research consistently reveals enlarged error-related negativity (ERN), a key EEG component measuring error monitoring in cognitive tasks that is located in the ACC [27, 48]. Furthermore, as with fMRI studies, EEG research has demonstrated substantially altered connectivity between networks reflecting inefficient neural processing with HTA. While there are indications that there is reduced connectivity between DMN and attentional control networks, the greatest effect of anxiety appears to be on connectivity between FPN and CON possibly contributing to dysfunctional updating of top-down attentional processes.

1.1.3.3.Evidence from Proton Magnetic Resonance Spectroscopy

Lastly, a less frequently used neuroimaging method in people with HTA is Proton Magnetic Resonance Spectroscopy (^{1}H-MRS). ^{1}H-MRS is a non-

invasive technique in which a single voxel of interest is selected within the brain to acquire a spectral profile. This spectral profile is based on hydrogen protons that have characteristic properties dependent on the molecules they are in. Hence local concentrations of different metabolites (typically neurotransmitters) can be quantified in arbitrary units (see [84] for further detail). It is common practice to conduct ^{1}H-MRS at rest to quantify neurotransmitter concentrations at a single point in time, while more recently studies have used functional ^{1}H-MRS to distinguish recruitment of neurotransmitters during task [85].

The neurochemistry of attentional control and how it is affected by trait anxiety and worry are currently poorly understood. Whilst some work has been conducted, investigating the relationship between anxiety and other neurotransmitters such as gamma-Aminobutric acid (GABA; [86]), the glutamatergic system has been researched to a greater extent in anxious cohorts. Glutamate (Glu), the brains primary excitatory neurotransmitter, is also known to be important for attentional control [87]. It acts on two families of receptors N-methyl-D-aspartate receptors (NMDAR) and AMPA and kainite receptors [88]. Of note, NMDARs are a frequent target for the pharmacological treatment of psychopathologies and related cognitive deficits [89]. Recent work in experimental animals reveals the importance of glutamatergic function for cognitive control. Jett and colleagues [90] showed in rats that impaired Glu neurotransmission in the Frontal Cortex may account for worse performance in an attentional shifting task. Furthermore, there is evidence in mice with genetically

compromised metabolisms that blockage of excess Glu receptors can improve cognitive impairments [91].

It has been demonstrated in animals as well as in humans that PFC Glu is highly affected by stress and stress-related psychopathologies, which in turn may affect cognitive control [92, 93]. For example, severity of social anxiety symptoms has been found to be associated with levels of Glu in the ACC, [94]. However, there is little research in non-clinical populations investigating the impact of trait anxiety on Glu levels. Furthermore, findings are mixed, while some studies found no differences in Glu levels between HTA and LTA participants [95], others report increased cortical Glu levels in participants with HTA compared to LTA [96].

Evidence is beginning to emerge showing that the glutamatergic system plays an important role in functional brain networks and their interaction; i.e. maintaining functional correlations and anticorrelations between these functional networks. Anticevic and colleagues [97] report that disrupting Glu transmission affects network function and interactions, in particular in the FPN, during a working memory task. Other research report similar effects of pharmacologically altering the glutamatergic system; reduced Glu concentrations are associated with reduces activation in the FPN during an attentional task [98]. In addition, Yücel and colleagues [99] show an altered relationship between ACC activation during cognitive control in opiate dependent subjects, who have reduced Glu concentrations in the Frontal Cortex. Finally, in a study combining ^{1}H-MRS and fMRI, Falkenberg and colleagues [87] demonstrate how Glu levels in the ACC

predict activation during cognitive control, indicating that the mechanism of how the brain implements cognitive control is related to Glu.

^{1}H-MRS, especially in combination with fMRI, has great potential to gain a more in-depth understanding of the functional and neurochemical processes underlying attentional control and how these processes are affected by trait anxiety. If the excitatory neurotransmitter Glu is altered in HTA individuals, and the glutamatergic system is important for effective brain function and associated cognitive performance, more work is needed to understand this mechanistic relationship.

1.2. **Real-time fMRI-Neurofeedback Training**

Neurofeedback allows the systematic self-regulation of brain activation in real-time. While EEG-neurofeedback is widely established in research and therapy [100], real time fMRI-neurofeedback (rt-fMRI-nf) is a relatively recent development in neuroscience. Rt-fMRI-nf allows participants to monitor and self-regulate their own brain activation during fMRI scanning and has an advantage over EEG-neurofeedback in offering much higher spatial resolution and specificity [101]. Feedback is traditionally provided visually, but can in principle be in any sensory modality. Usually, one or more target region(s) are predefined based on anatomical landmarks and/or brain activation elicited by a functional localiser task. There are also whole-brain approaches to rt-fMRI-nf, for example, based on multivariate pattern analysis (MVPA) that do not require a priory selection of target regions [102].

During traditional rt-fMRI-nf, brain activation, from the target region or network, is measured and fed back to the participant so they can monitor this and progressively achieve voluntary control over their own neural activation. Feedback displays are often adapted to the maximum absolute signal in individual subjects (e.g., [103]). The simplest approach for rt-fMRI-nf is providing feedback of the average brain activation in a single brain region – sometimes relative to activation during rest or in a pre-defined reference region. The participant is usually instructed to try to achieve a change in brain activity that is represented by changes in a visual display (e.g., a gauge that can be moved up or down based on changes in activation relative to maximum and minimum activation). More complex rt-fMRI-nf approaches target functional (e.g., [103, 104]) or effective connectivity [105] between multiple regions of the brain. Although the exact processes underlying rt-fMRI-nf learning are unclear, training effects due to rt-fMRI-nf are most often ascribed to operant conditioning [106, 107]. A recent review by Sitaram and colleagues outlines details of other models of learning that have been ascribed to EEG- and fMRI-neurofeedback [106].

The typical setup of a rt-fMRI-nf experiment requires real-time export of the reconstructed data acquired from the participants' brain from the MRI scanner to a separate analysis computer for immediate (online) analysis. The specific analysis components may vary between different experiments, but usually entail simple preprocessing of the data (e.g., motion correction, spatial smoothing) and depending on the specific requirements of the experiment a General Linear Model (GLM) may be

applied. This step can be computationally very intensive, which can produce a delay in feedback presentation. Therefore, the analysis computer needs to have sufficient capacity to perform online analysis, to minimise delays in feedback presentation. The result of online analysis is presented to the participant in the MRI scanner in real-time via a stimulation computer, which may or may not be the same as the analysis computer. Again, dependent on the requirements of the experiment the processed signal is translated into a feedback presentation (typically visual), that can be interpreted intuitively by the participant. Overall, this produces a closed-loop system, illustrated in

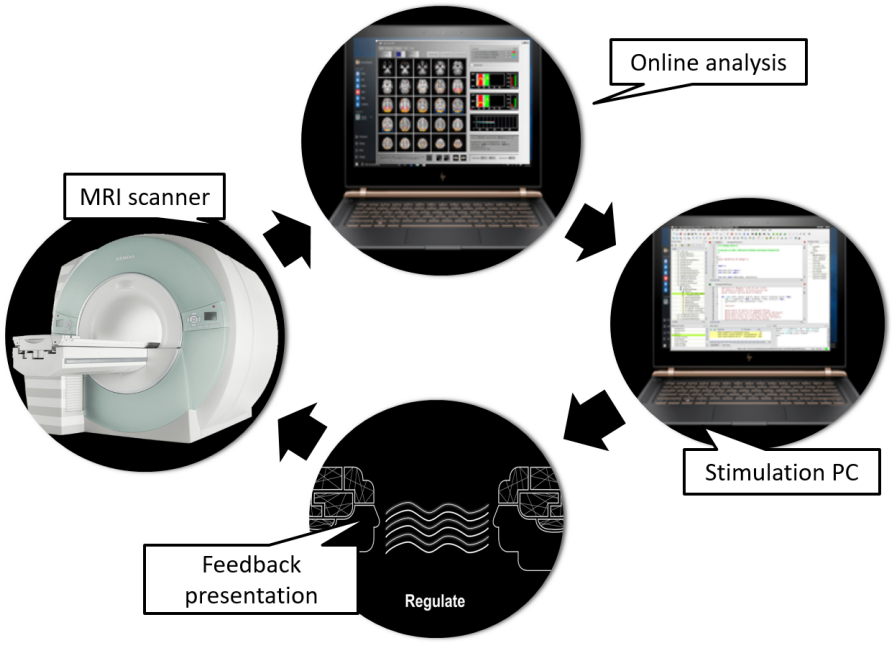

Figure *1*. The real-time nature of rt-fMRI-nf is essentially constrained not only by technology (e.g., computing power), but also by biology (e.g., the sluggish hemodynamic response). The effect of hemodynamic delay can be somewhat circumvented with intermittent rt-fMRI-nf (Section 1.2.3.6) or by providing feedback based on more than one MRI volume (e.g., [108]).

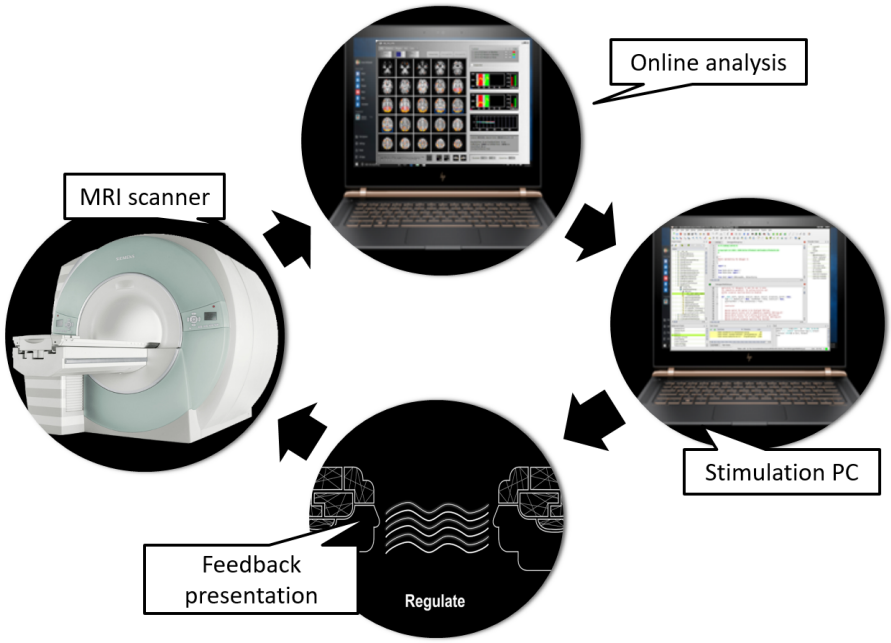

Figure 1. Setup of a typical rt-fMRI-nf experiment. Closed-loop between the MRI scanner, analysis computer, stimulation computer and feedback presentation.

Rt-fMRI-nf has been shown to lead to neural changes in the neurofeedback target region and network. Besides these effects, that are specific to the target region, Emmert and colleagues [109] demonstrated in a meta-analysis, combining the results of eight neurofeedback studies in healthy subjects, which used various target regions and directions of regulation, that there is a general pattern of brain activation associated with rt-fMRI-nf self-regulation effort (i.e. the activity of self-regulation). Rt-fMRI-nf training is generally associated with increased activations in the Posterior ACC, bilateral Anterior Insula Cortex, bilateral Ventro-Lateral PFC, bilateral DLPFC, bilateral Temporo-Parietal regions, bilateral Parietal regions, bilateral Occipital and various regions throughout the Basal

Ganglia and Thalamus. In contrast, the Precuneus, PCC, bilateral Temporal Transvers and right Parietal regions show decreased activation during rt-fMRI-nf training. In effect, this data does not take into account the area of regulation or regulation success. This pattern of increased neural activation during rt-fMRI-nf regulation is topologically similar to the EMN, while the areas that are deactivated during rt-fMRI-nf regulation are predominantly part of the DMN [35]. Regions with increased activation during rt-fMRI-nf regulation, as described in Emmert and colleagues [109], include large parts of the FPN, CON and VAN; in addition to occipital regions important for visual processing.

1.2.1. *Neurofeedback Based on Amplitude of Brain Activation*

A common design in rt-fMRI-nf studies is to provide participants with feedback based on the relative amplitude of brain activation in one defined target region. The implementation of relative amplitude differs between experiments; brain activation may be scaled to the range of activation measured in a localiser task relative to activation during rest periods and/or relative to activation in a nuisance region of interest (ROI) that is not expected to be activated by a particular task. Ruiz and colleagues [110] provide a detailed overview of rt-fMRI-nf studies on regulation of a single target region published between the years of 2002 and 2013. In addition Thibault and [111] colleagues provide a critical review of 99 experimental rt-fMRI-nf studies published between 2004 and 2017, most of which are studies where feedback is given based on amplitude of activation in a single target region. Overall these reviews show that, in most cases, participants can learn to gain control over the amplitude of brain activation

in single brain regions when veridical feedback is provided. However, in transfer runs without feedback and in behavioural outcome measures results are at best mixed. Notably, studies frequently did not test for effects beyond activation during self-regulation [111]. It is important to consider that while rt-fMRI-nf on single brain regions is the simplest possible design, it is not necessarily reflective of the complexity of how the brain functions [110]. Nevertheless, studies have documented that during rt-fMRI-nf regulation of activation in a single target region, functional connectivity between the rt-fMRI-nf target region and its wider network is altered [112-115]. Consistent with this, other studies which examined changes in effective connectivity also report changes in wider network connectivity during and after rt-fMRI-nf training of a single target region [113, 116]. In addition to rt-fMRI-nf designs based on the amplitude of activation in one target region, there are more complex designs based on amplitude of brain activation in two or more brain regions (e.g., [117-121]).

1.2.2. *Neurofeedback Based on Measures of Connectivity*

More recent rt-fMRI-nf studies have utilised feedback based on different connectivity measures of the brain. These can be broadly categorised in three groups; those using a correlation-based approach, those using multivariate pattern analysis (MVPA) and those using dynamic causal modelling (DCM).

Correlation-based rt-fMRI-nf is either implemented using a sliding-window for continuous feedback (e.g., [103, 122]) or a design with end-

of-block feedback when intermittent feedback presentation is used (e.g., [104]). The benefits of continuous vs. intermittent feedback presentation have been outlined elsewhere and are equally applicable to correlation-based rt-fMRI-nf (Section 1.2.3.6). Sliding windowed correlation provides a dynamic measure of functional connectivity based on the correlation between measures within a fixed-length moving time window (i.e. sliding window). The length of the sliding window is hereby partly dependent on the desired effect, while there is a trade-off between decreased Signal to Noise Ratio (SNR; longer window) and increased dynamicity (shorter window) of the feedback signal. Rt-fMRI-nf targeting functional connectivity between brain regions using sliding-windowed correlation has been demonstrated as superior to activation-based measures in representing task-related activation [123]. However, the decision for a rt-fMRI-nf target should always be dependent on the desired outcome of rt-fMRI-nf training. There are variations of correlation-based rt-fMRI-nf, for example in a study by Kim and colleagues [124] in an intervention to reduce cigarette cravings, rt-fMRI-nf was provided based on a combination of amplitude *and* connectivity information. The study reports more successful self-regulation, when connectivity information is included in the calculation of the feedback score [124].

MVPA-based rt-fMRI-nf or decoded neurofeedback (dec-nf) has gained popularity in recent years. Dec-nf uses functional localisers and different classifier methods to subsequently provide custom feedback on relevant brain regions [125]. This method is highly customised, which may be especially beneficial in a clinical context. However, it is susceptible to

confounding factors and less useful when there is a very specific neurocognitive model of the underlying neural process to be modified. Dec-nf is also not suitable to test hypotheses on underlying processes as MVPA as a process is driven by statistical rather than theoretical considerations. Initial studies show that it is feasible for participants to gain control over activation patterns with the aid of Dec-nf (e.g., [102, 126]).

Correlation-based rt-fMRI-nf and Dec-nf are both based on measures of functional connectivity, which are derived from temporally synchronised patterns of activity in spatially distinct regions of the brain. Functional connectivity however, does not provide information about directionality or causality of these correlational relationships. In contrast, DCM is a non-linear Bayesian technique to measure effective connectivity, which allows for directional predictions. Hence, DCM allows for the prescription of causal relationships in neural networks [127]. Only few studies have employed this approach for rt-fMRI-nf training [105, 128]. DCM relies on very complex analysis procedures and requires more time for computation compared to other connectivity-based methods [123]. This poses constraints on feedback presentation when using DCM-based rt-fMRI-nf. Current studies have provided near-real-time feedback after blocks of regulation (intermittent feedback), rather than dynamically changing feedback displays (continuous feedback). Nevertheless, participants in DCM-based rt-fMRI-nf studies have been able to successfully self-regulate brain activation [105, 128] and DCM-based rt-fMRI-nf has superior specificity to other methods for rt-fMRI-nf [129].

While all of the aforementioned methods of connectivity-based rt-fMRI-nf have produced positive results in initial studies, they are still being developed, so there is currently no established method or consensus on how to best modulate connectivity using rt-fMRI-nf.

1.2.3. *Common Design Choices in rt-fMRI-nf*

Currently, rt-fMRI-nf as a method is still under development. There is no one established method or design for rt-fMRI-nf experiments. This section will address seven considerations for optimal outcomes when designing rt-fMRI-nf studies; namely the choice of experimental control (Section 1.2.3.1), target region for regulation (Section 1.2.3.2), direction and magnitude of feedback (Section 1.2.3.3), modality of feedback presentation (Section 1.2.3.4), instructions to participants (Section 1.2.3.5), continuous or intermittent feedback presentation (Section 1.2.3.6) and transfer runs and pre- and post-training measures (Section 1.2.3.7).

1.2.3.1.Experimental Control

As in any experimental study, it is important to control for extraneous variables and to demonstrate the benefit of rt-fMRI-nf training in changing brain activation and transfer outcomes compared to other methods. Sorger and colleagues [130] provide a detailed overview on control conditions for rt-fMRI-nf. Currently, research primarily relies on placebo controls (e.g., feedback from non-target signals or sham feedback) and some studies have used bidirectional regulation control groups, in which participants train to self-regulate the same target as experimental participants, but in opposite direction. Few experiments have compared more than one control group

and there is currently no experimental evidence for substantial differences in efficacy between control groups. The need to control for specific extraneous variables can differ depending on study aims and not all types of control are always feasible or ethical. In the current stage of development of rt-fMRI-nf, establishing the specific efficacy of neurofeedback by controlling for effects of placebo and non-specific effects of the intervention seems most appropriate. In this context it is especially important to account for the experience of self-regulation as previous rt-fMRI-nf studies have suggested that this may play a major part in the therapeutic effect of training [131, 132]. While there is no consensus over the ideal choice of control group some studies have employed more than one control group [133, 134].

When considering rt-fMRI-nf as a neurotherapeutic intervention in clinical contexts it is important to evidence the incremental benefit of this intervention compared to treatment as usual. Currently, there have not been clinical trials in which rt-fMRI-nf has been compared to treatment as usual control; however, this approach is part of the recent consensus on the reporting and experimental design of clinical and cognitive-behavioural neurofeedback studies (CRED-nf; [130, 135]). Nevertheless, some efforts have been made in comparing rt-fMRI-nf to other techniques. For example, in deCharms and colleagues [133], a control group was employed that trained with biofeedback from non-brain sources in order to alleviate chronic pain. Furthermore, an ongoing clinical trial if rt-fMRI-nf for alcohol dependence is using treatment as usual as a control [136].

An alternative to between-subjects control conditions are internal or within-subjects controls. While some argue that under most circumstances these do not constitute a suitable alternative to between-subjects controls in rt-fMRI-nf studies [130], Marxen and colleagues [137] conclude, that internal controls are more appropriate (i.e. rather than employing a separate control group), due to theoretical consideration. Indeed, it is not uncommon for rt-fMRI-nf studies to employ internal control conditions (e.g., [122, 137-141]). Internal control conditions can be based on counterbalanced repeated measures designs [122], which raise questions about the long-term and carry-over effects of rt-fMRI-nf training. Nevertheless, there are more sophisticated approaches such as including both up- and down-regulation of the target region in the study design [137]. However, this approach may not always be possible or ethical (e.g., in clinical settings). Having conditions of up- and down-regulation in experimental rt-fMRI-nf studies potentially helps ruling out general effects of self-regulation that can be a confounding factor when evaluating regulation success [109, 137].

1.2.3.2.Target Region for Regulation

The target region for regulation in rt-fMRI-nf studies is almost always a question of the desired cognitive or clinical outcome of training. Some studies have compared the efficacy of rt-fMRI-nf in different target regions, concluding that some regions are easier to regulate for participants than others [142, 143]. Importantly, one must consider the influence of confounding factors, such as activation associated with regulation effort overlapping with specific target regions. In addition, pilot work is an

important step in the planning of rt-fMRI-nf experiments to ensure the suitability of the desired target region. Furthermore, the size of target region (along with other factors) has an effect on SNR and consequently the reliability of the feedback signal [144]. Currently, it is not common practice to report the size of target region in rt-fMRI-nf studies, despite this being an important variable in the experimental design of a study.

Most rt-fMRI-nf studies use a combination of anatomical landmarks and a functional localiser scan to define target regions (e.g., [145, 146]), other studies purely rely on anatomical landmarks only (e.g., [147]). Once defined, the target region for rt-fMRI-nf training usually remains the same, but can be adjusted dependent on regulation success to fit participants' needs and to achieve better results (e.g., in clinical studies; [148]). Furthermore, depending on the specific aim of a rt-fMRI-nf experiment target regions for regulation may vary significantly between participants (e.g., [149]).

All rt-fMRI-nf techniques but MVPA-based rt-fMRI-nf require a priori defined target regions. Studies using novel approaches, including rt-fMRI-nf targets that have not been validated in previous work, are recommended to establish a reliable signal from the target regions used and adjust their design if required [144].

1.2.3.3.Direction and Magnitude of Feedback

In traditional rt-fMRI-nf designs, participants are typically instructed to up-regulate activation of one or more brain regions to maximise activation with the aid of feedback. A minority of studies use down-regulation (e.g.,

[150, 151]) of brain regions to minimise activation, while ultimately the choice of direction of regulation is subject to the desired effect of rt-fMRI-nf on behavioural and brain activation.

Some studies employ a combination of up- and down-regulation, such as Marxen and colleagues [137] or deCharms and colleagues [133]. Using both blocks of up- and down-regulation may be most suitable for participants to learn to control activation in a specific region. It also provides participants with the opportunity to experiment with mental strategies and explore their control over activation in more than one direction. This method can also be used to provide an internal control of regulation success. However, it comes with increased complexity and ethical considerations, depending on the expected effect of up-/down-regulation, if either one of them is expected to be detrimental to the participants (e.g., in clinical settings).

Typically rt-fMRI-nf regulation is to the maximum activation/deactivation. However, graded rt-fMRI-nf is an emerging technique for participants to attain greater control over the target region. This approach is yet very novel and has produced mixed results (e.g., [146, 152]).

While most studies have been based on up- and down-regulation of activity in one or more brain regions, if there is no simple relationship between activation of a brain region and the target process, more complex designs such as connectivity-based rt-fMRI-nf may be used for optimal outcomes (see 0).

1.2.3.4.Modality of Feedback Presentation

By far the most common modality used for feedback presentation in rt-fMRI-nf studies is visual. Visual feedback can be presented in different forms, such as thermometer and line graphs [112, 141, 153] or more complex visual displays (e.g., fire [133], rocket [131, 154], integrated in task [102]). Other non-visual modalities used are auditory [155, 156], sensory stimulation [150], social [157] or monetary reward [104, 158, 159]. Interestingly, both social and monetary reward achieved higher regulation success than a control group with no monetary reward [157, 159] reinforcing the theory that rt-fMRI-nf learning is in fact a form of operant conditioning.

While typical feedback presentation is a representation of brain activation, DeBettencourt and colleagues [102] employed an original design in which MVPA-based rt-fMRI-nf was implemented by manipulating task difficulty rather than a separate feedback display in response to changes in brain activation. This is not only a unique way of feeding-back information on the participants brain states, but simultaneously participants were rewarded with an easier task when they achieved more desirable brain states.

To date there has been no systematic comparison between the effectiveness of feedback from different modalities and there is no strong reason to suggest differences between them. Nevertheless, under practical considerations visual feedback presentation may be the easiest modality to implement under the constraints of the MRI scanner setting. However,

using for example auditory stimuli for feedback presentation can be more appropriate for specific patient populations (e.g., visuo-spatial neglect [156]).

1.2.3.5.Instructions to Participants

Most rt-fMRI-nf studies provide participants with specific instructions on mental strategies to regulate brain activation [111]. Though some studies with explicit strategies have demonstrated the effectiveness of this approach (e.g., [114, 160]), there is no conclusive evidence whether providing participants with explicit strategies for self-regulation improves learning. This may well depend on the region that is regulated and the process that is to be improved [107]. Not providing explicit strategies may be advantageous, as this does not limit the participants' ability and freedom of finding a personal strategy, and encourages implicit learning from the feedback signal [137]. Sepulveda and colleagues [159] showed in a study aiming to up-regulate supplementary motor area (SMA) activation, that instructing the use of an explicit strategy (motor imagery) did not facilitate successful regulation, while uninstructed regulation was successful. Marxen and colleagues [137] specifically did not give any instructions to participants to test whether participants were able to regulate brain activation without explicit strategies and concluded that this was possible. More research is needed to establish whether it is beneficial for self-regulation of activity if explicit strategies are provided, but critics have noted that if a successful mental strategy for self-regulation was known the incremental benefit of rt-fMRI-nf training may be minimal if any [111].

1.2.3.6.Continuous or Intermittent Feedback Presentation

Most rt-fMRI-nf studies are using continuous feedback, which is updated after each volume with a delay of a few seconds due to the sluggish hemodynamic response and computing time. Providing participants with intermittent neurofeedback (i.e. end of block feedback) may be a suitable way to accommodate hemodynamic delay and improve SNR by averaging several volumes [144]. It may also be advantageous to reduce cognitive load for participants [153]. However, some studies show that intermittent feedback is only advantageous for single-session experiments, while continuous feedback achieved better regulation results long-term [161]. Furthermore, erroneous strategies may be corrected more quickly and not shape behaviour as much with continuous feedback [102]. Overall effect sizes for the differences between intermittent and continuous feedback are small. Notably the choice of feedback information can limit the option of giving continuous feedback to participants (e.g., when using computationally expensive techniques, such as DCM-based neurofeedback; [105]).

There is no conclusive evidence as to which mode of rt-fMRI-nf is better. The choice between intermittent and continuous feedback will most likely be based on theoretic considerations and technical limitations.

1.2.3.7.Transfer Runs and Pre- and Post-Training Measures

Transfer runs are used to demonstrate that are able to maintain regulation of brain activation in the absence of feedback. Different methods of transfer run have been employed, for example runs very similar to rt-fMRI-

nf runs, but without feedback [114, 117, 134, 147, 153] or transition from continuous to intermittent to no feedback modes [137]. For instance, Marxen and colleagues [137] used a sophisticated design with a gradual progression over neurofeedback sessions from continuous rt-fMRI-nf, to end of block feedback and finally transfer runs, during which no feedback was given. Rt-fMRI-nf studies using transfer runs have demonstrated that participants can maintain self-regulation of brain activation beyond rt-fMRI-nf training (e.g., [137]). However, successful self-regulation in transfer runs is not consistently reported (e.g., [147]).

Pre- and post-training measures differ from transfer runs, with respect to how the success of rt-fMRI-nf training is measured. While transfer runs are used to demonstrate continued regulation ability, pre- and post-training measures are to demonstrate rt-fMRI-nf-induced changes in activation patterns during task or rest or changes in behaviour or symptomatology.

Resting-state fMRI is a valuable pre- and post-measure of rt-fMRI-nf interventions, as it provides a whole brain perspective of changes in neural circuitry. Resting-state functional connectivity (RSFC) has been successfully altered with rt-fMRI-nf on a single brain region as well as with rt-fMRI-nf based on functional connectivity [104, 141, 162, 163].

A less common alternative to resting-state fMRI as a pre- and post-training measure is using relevant tasks to establish whether brain activation is altered during those tasks as a result of rt-fMRI-nf training. For instance, Hui and colleagues [112] used two different motor tasks and compared activation and connectivity during these tasks pre- and post-training

between groups, showing that task-related activation is correlated with the rt-fMRI-nf training effect.

Some studies only test behavioural effects of rt-fMRI-nf (e.g., [102, 145]. Evaluation of relevant changes in behaviour pre- and post-rt-fMRI-nf is an important outcome measure, however it is best paired with additional measures that measure changes in brain activation. Similarly, some studies rely on questionnaire outcomes or symptom change as main outcome measure (e.g., [109]), these outcome measures are important especially in clinical work. Nevertheless, transfer runs or other pre- and post-evaluations of changes in brain activation are needed in addition to secure the relationship between symptom changes and altered brain activation.

1.2.4. *Applications of rt-fMRI-nf*

Using rt-fMRI-nf, participants can learn to regulate amplitude of brain activation in one or more target regions, or to regulate connectivity between brain regions. There are three main applications of rt-fMRI-nf: clinical intervention, cognitive enhancement and scientific discovery.

By far the most frequent application of rt-fMRI-nf to date is as a clinical intervention. When changes in localised or network brain activation have been clearly linked to mental disorders or symptoms, there may be unique benefits for patients if they can learn to regulate these targeted brain activation patterns with rt-fMRI-nf training. Even when there is no clear link between altered activation/connectivity and psychopathology, Dec-nf can be used to identify and modulate maladaptive activation patterns (e.g., [164]). The first studies examining the potential of rt-fMRI-nf for clinical

use have reported optimistic findings in depression [148, 163, 165], post-traumatic stress disorder (PTSD; [114, 141]), chronic pain [133, 166] schizophrenia [118, 154] and others disorders [122, 167, 168]. These findings are promising, especially for patients who are unresponsive to other treatment options. Typically, these interventions target prominent alterations in brain activation observed in the respective disorder (e.g., amygdala activation in PTSD; [114]). Alternatively, they target specific symptoms that are characteristic of the disorder (e.g., auditory hallucinations in schizophrenia; [154]). Much work is yet to be done in the development of rt-fMRI-nf as a credible clinical tool. In particular, it is important to demonstrate its benefit over other interventions (i.e. treatment as usual) and its potential in combination with traditional treatments. The duration of symptom improvements brought about by rt-fMRI-nf training also need to be better established. Nevertheless, there are reasons to be optimistic that rt-fMRI-nf can add to existing interventions, in particular for patients in whom traditional treatments have been unsuccessful.

Another application of rt-fMRI-nf is for cognitive enhancement. Scharnowski and Weiskopf [169] provide an extensive review of studies aiming to enhancement cognitive functions using rt-fMRI-nf. While effect sizes are small and training is not successful in all participants, rt-fMRI-nf has been shown to be a feasible method to alter visual perception, motor control, working memory, linguistic processing and emotion processing [169]. Further studies confirm the use of rt-fMRI-nf for cognitive enhancement, for instance, to improve working memory [145, 170] and attention [102]. Frequently, studies aiming at cognitive enhancement

report altered brain activation and connectivity, but do not test for (e.g., [171]) or do not measure behavioural improvements (e.g., [145, 172, 173]).

While rt-fMRI-nf has potential as a clinical tool and for cognitive enhancement, it also opens opportunities for scientific discovery. Rt-fMRI-nf with its unique properties of modifying neural circuitry has large potential for establishing causal mechanisms in neurocognitive research. The traditional approach of experimental research will either manipulate the environment or cognitive state of participants to evaluate changes in brain activation; however, this approach only allows associative inferences to be made. Alternatively, populations with altered brain activation are selected for correlational research of how symptomatology is linked to altered brain activation. Rt-fMRI-nf allows for experimental manipulation of brain activation, and the ensuing effects on behaviour or symptoms can then be assessed, assuming a suitable experimental design is employed. This allows more causal inferences to be established. However, this is currently not a focus of rt-fMRI-nf research.

1.3. Outline and Objectives of Study

The overall aim of the research presented in this thesis is to investigate and better understand the neural processes underlying impaired attentional control in people with HTA and to investigate the feasibility of DLPFC-ACC functional connectivity-based rt-fMRI-nf training to improve attentional control and anxiety levels. To this end two separate studies have been conducted. Firstly, an MRI correlational study combining ^{1}H-

MRS and fMRI measures was conducted to investigate the relationship between brain activation and Glu levels and how these are affected by trait anxiety (Chapter 2). Secondly, a randomised controlled experiment using rt-fMRI-nf based on the functional connectivity between DLPFC and ACC was used in an attempt to manipulate activation in brain regions and networks important for attentional control. This study included several pre- and post-training measures that are reported in separate experimental chapters (Chapters 3-6).

The first study, which combined ^{1}H-MRS-fMRI, it was hypothesised, that levels of trait anxiety would be positively associated with DLPFC activity during a cognitive control task (indicative of *processing inefficiency*). Furthermore, based on previous findings, it was predicted that participants with HTA would show elevated levels of PFC Glu relative to a LTA group. Finally, the association between resting-state PFC Glu levels and DLPFC activity during cognitive control and how this was affected by trait anxiety was investigated.

For the rt-fMRI-nf experiment, it was hypothesised that in HTA individuals, rt-fMRI-nf training of DLPFC-ACC functional connectivity would increase functional activation and connectivity in DLPFC and ACC regions. It was further predicted that these neural changes would be associated with reduced anxiety levels, as reduced DLPFC-ACC functional connectivity has been associated with anxiety. It was then investigated if the effects of rt-fMRI-nf training would transfer to improve attentional control during offline behavioural tasks measuring inhibition,

sustained attention and attentional bias to threat. Finally, it investigated whether if rt-fMRI-nf training would alter RSFC network interactions between attentional control networks and the DMN. Specifically, that the anticorrelation between these networks would increase, as it has been shown to be decreased in people with HTA.

2. Altered Relationship Between Prefrontal Glutamate and Activation during Cognitive Control in People with High Trait Anxiety

(Adapted from published paper, published in Cortex under the title "Altered relationship between prefrontal glutamate and activation during cognitive control in people with high trait anxiety"; Appendix 1)

2.1. Introduction

Trait anxiety is a normally distributed personality dimension and a risk factor for anxiety and depressive disorders [3, 174] characterised by intrusive thoughts, worry and difficulty in disengaging from negative material [2]. Trait anxiety has been found to be associated with functional consequences including increased distractibility and attention problems [5-7]. Indeed, the effects of trait anxiety on cognitive function have long been recognised [17] and are accounted for by attentional control theory (ACT; [7, 21]).

ACT proposes that anxiety competes for attentional resources and impairs cognitive control when executive processes are required, i.e. updating, set shifting and inhibiting irrelevant or distracting information. Consequently, anxiety can impair task performance i.e. *performance effectiveness* when executive control is required. Further, ACT predicts that, even when *performance effectiveness* is maintained, anxiety can reduce *processing efficiency* (the quality of performance relative to use of processing or cognitive resources). In line with this prediction, functional fMRI studies

report increased PFC activation in people with HTA without concomitant improvements in *performance effectiveness* (i.e. *processing inefficiency*; [13-15]). The PFC along with the lateral parietal cortices i.e. the FPN, are known to be important for cognitive control [36, 37] and support 'top-down' attention by maintaining attentional sets [37-39]. In particular the DLPFC, comprising the middle and superior frontal gyri, has a central role in top-down cognitive control [175] and has been shown to have altered activation in response to tasks that require cognitive control in people with HTA (e.g., [6, 13-15]).

Despite these recent advances in the understanding of the neural mechanism involved in cognitive control, little is known about its neurochemistry and how this may be affected by individual differences in trait anxiety. Glutamate (Glu) is an excitatory neurotransmitter and its importance in cognitive control has been highlighted in animal models [90, 91]. In humans, Anticevic and colleagues [97] showed that administration of ketamine, an N-methyl-D-aspartate glutamate receptor (NMDAR) antagonist, disrupts activity in FPN regions and subsequent performance during a working memory task, highlighting the role that Glu plays in cognitive control. Combining functional Magnetic Resonance Imaging (fMRI) and ^{1}H-Magnetic Resonance Spectroscopy (^{1}H-MRS), Falkenberg and colleagues [87] demonstrated that the magnitude of the blood-oxygen level-dependent (BOLD) response to a task requiring cognitive control was predicted by anterior cingulate resting-state Glu levels. Moreover, individual variability in resting-state Glu levels was related to how the brain implements cognitive control [87].

These findings are important because Glu functioning is altered in some psychiatric disorders associated with cognitive control impairments [89] and pharmacologically induced reductions in Glu levels have been found to alter the BOLD response during cognitive control tasks [98, 99]. However, whilst in vivo ^{1}H-MRS studies investigating the neurobiology of anxiety have focused on populations with diagnosed disorders (e.g., [176-179]), ^{1}H-MRS studies in non-clinical populations in which trait anxiety is assessed as a personality dimension are relatively few in number. The first study using ^{1}H-MRS to examine metabolite levels in relation to trait anxiety reported increased PFC N-Acetyl aspartate (NAA) in participants with HTA but found no differences in Glu levels between HTA and LTA participants [95]. More recently, Modi and colleagues [96] reported that cortical Glu and combined Glu and glutamine levels (measured with ^{1}H-MRS in the anterior cingulate) were increased in participants with HTA relative to LTA scores and predictive of trait anxiety levels across their study cohort. Pharmacologically induced anxiety has also been reported to increase cortical Glu levels [180].

Together, the studies discussed here indicate that trait anxiety can affect both DLPFC activity during cognitive control and PFC Glu levels. Whilst it has already been established that resting-state cortical Glu levels are important for the way the brain implements cognitive control [87, 97], to date, no studies have measured resting-state cortical Glu levels and DLPFC activity during a cognitive control task and examined how these are related to individual differences in trait anxiety levels. This is important because it is possible that the effects of trait anxiety on DLPFC activity

(and cognitive control) are influenced by cortical Glu levels. Although the precise relationship between resting-state PFC Glu levels and neural activity is not fully understood, a number of studies have shown that levels of resting-state Glu measured with ^{1}H-MRS are related to the BOLD signal and electrophysiology measures during cognitive tasks [87, 181-183] and possibly mediated via NMDAR [97].

The aim of the present study was to investigate the relationship between trait anxiety, PFC Glu levels (using ^{1}H-MRS) and activity in DLPFC during a cognitive control task. In accordance with the predictions of ACT and findings from previous fMRI studies, it was hypothesised that trait anxiety would be associated with decreased performance in and increased DLPFC activity during a cognitive control task (indicative of *processing inefficiency*). Based on the findings outlined above, it was then tested if participants with HTA had elevated levels of PFC Glu relative to a LTA group. Finally, it was explored how the association between resting-state PFC Glu levels and DLPFC activity during cognitive control was affected by individual differences in trait anxiety levels.

2.2. **Methods**

No data were excluded and inclusion/exclusion criteria are reported below. Inclusion/exclusion criteria were established prior to data analysis as were all manipulations, and all measures in the study. The raw data and materials to replicate this study or any analysis are available at Open Science Framework (DOI 10.17605/OSF.IO/PXK8Z).

2.2.1. *Participants and Assessments*

Thirty-nine participants performed a colour-word Stroop task [184] while functional magnetic resonance imaging and ^{1}*H-MRS* data were acquired. Participants (27 female) ranged from 18-37 years of age (M = 22.05 years, SD = 4.62). There were 35 right handed and four left handed participants, as assessed by the Annett Hand Preference Questionnaire [185]. Participants were recruited from the University of Roehampton, Royal Holloway University of London and from the general public. Participants had no prior neurological or medical illness and were not using medication for anxiety or depression. The University of Roehampton Ethics Committee granted ethical approval and all participants gave written informed consent prior to taking part in the study (Appendix 2.1.). IQ was estimated using the Wide Range Achievement Test Reading Level 2 [186]; M = 109.15 (SD = 10.24, Range 86-131) to control for potential effects of IQ on task performance and task-related BOLD signal. Alcohol consumption and recreational cannabis use were assessed for all participants using a categorical scale (ranging from no-use to regular use). The majority of participants indicated that they used alcohol on a moderate basis and that they used cannabis never or only experimentally (Table 1).

Table 1

Frequency of alcohol and cannabis consumption across participants.

	No or experimental use	Occasional use	Moderate use	Regular and severe use
Alcohol	4	21	12	2
Cannabis	35	2	2	0

To assess trait anxiety, participants completed the State Trait Anxiety Inventory (STAI) [4]. In all participants the mean score for trait anxiety was 41.33 (SD = 11.07, Range 22-78) and 33.2 (SD=10.01, Range = 20-70) for state anxiety. This distribution of STAI trait scores is slightly higher than published norms (i.e. M = 36, SD = 10; [4]) but comparable to scores reported by a previous study examining effects of trait anxiety on DLPFC activation (i.e. M = 43 SD = 11; [6]).

A median-split of STAI trait scores was used to establish LTA (n = 19, 6 male, 2 left-handed) and HTA (n = 20, 6 male, 2 left-handed) groups (Section 2.3.1.), this dichotomization was performed to achieve greater interpretability of the results. Confirmatory analysis of behavioural and MRI data using STAI trait scores as a continuous variable are reported in Appendix 3.

2.2.2. *Experimental Task*

Participants performed a colour-word Stroop task adapted for MRI and used previously [187]. The task was programmed and presented with Microsoft Visual Basic. Participants responded with one of four fingers of their right hand to the font colour of the word presented (Red, Yellow, Blue or Green). Participants were instructed to respond as quickly and as accurately as possible while RT and ER were recorded. The task consisted of a total of 100 trials, 33 congruent trials in which the font colour and meaning of the word matched, 33 incongruent trials in which the font colour and meaning of the word did not match and 34 fixation periods in which the participants saw a fixation cross. Trials were presented in a

pseudo-randomised order within one functional run lasting 10 minutes. Each trial (including fixation cross trials) was presented in the middle of the screen and took 6000 ms including a period of 1300 ms before trial onset in which a blank dark grey screen was displayed. Participants then viewed a visual stimulus (i.e. congruent word, incongruent word, or fixation cross) that was presented for 700 ms. Thus participants were allowed 4700 ms from stimulus onset (700 ms during trial presentation plus 4000 ms response period) to respond i.e. responses were registered from the onset of each stimulus trial. After a response was registered the trial continued until the end of this period. No response was required in fixation cross trials.

2.2.3. *Power Calculations*

To test if analyses were sufficiently powered, G*Power (https://download.cnet.com/G-Power/3000-2054_4-10647044.html) was used. The power calculations suggest that, with independent group sizes of n = 19 (LTA) & 20 (HTA), the study would only have sufficient power to detect a significant group difference (using an independent sample t-test) in DLPFC activity if the effect size was >.8 (large). Thus, the sample size is insufficient to detect small and medium effect sizes. However, based on the effect size of 0.49 reported by Bishop [6] for a significant positive correlation between STAI trait anxiety scores and DLPFC activity (see [6], Figure 2c), the power calculation show that an n = 36 has > 90% power to detect a significant positive association between STAI scores and DLPFC activation at p =.05 (one-tailed). As the n = 39 for this analysis, it can be assumed that it is sufficiently powered.

To this day only one previous study has reported differences in PFC Glu levels (in the ACC) for HTA vs. LTA groups [96]. This study reports an effect size (Cohen's d) = .85, but is based on a small sample. Generically, using mean and standard deviation data from an independent ^{1}H-MRS Glu dataset [188] an effect size for PFC Glu levels based on a small to medium (15%) change in Glu levels between groups (Cohen's d) = .90 was calculated. Using an intermediate effect size = .875 a power calculation shows that n = 19 has >85% power to detect a significant independent group difference for PFC Glu levels at p = .05 (one-tailed).

2.2.4. *Statistical Analysis*

IBM® SPSS Statistics Version 22 was used for the analysis of task and questionnaire data. Questionnaire and task data were considered normally distributed. A multifactorial repeated measures ANOVA with the dependent variables RT and ER in the two conditions of the Stroop task (Congruent, Incongruent) was performed. Trait anxiety group was included as a between-subjects factor. A statistical significance threshold of $p < .05$ was applied throughout. Furthermore, the statistical software program JASP (JASP Team, 2016; jasp-stats.org) was used to compute Bayes Factor (BF_{10}) to quantify the relative likelihood of the model tested to the null hypothesis. LTA and HTA groups were compared on STAI trait and state scores, IQ estimate and age using independent samples t-tests. The groups were also compared on their alcohol consumption and cannabis use using Mann-Whitney U tests for ordinal data.

2.2.5. *MRI Acquisition*

All MRI scans were acquired on a 3T Siemens Magnetom TIM Trio scanner using a 32-channel head coil. Structural T1 weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MP RAGE) images were acquired with a spatial resolution of 1 mm × 1 mm × 1 mm, in plane resolution of 256 × 256 × 176 slices and scanning time of approximately 5 minutes. Functional images were acquired using a full-brain, anterior-to-posterior, T2* weighted, BOLD-sensitive gradient echo planar sequence with the following parameters: TR/TE/flip angle = 2 s/40 ms/70°, field of view 192 mm × 192 mm and slice thickness of 5 mm giving a voxel size of 3 mm × 3 mm × 5 mm and whole brain coverage of 28 interleaved slices. Three hundred volumes were collected during the event-related functional run.

2.2.6. *^{1}H-MRS Data Acquisition and Analysis*

^{1}H-MRS in vivo spectra were acquired from a 20 × 20 × 20 mm voxel located in the right medial PFC during rest. A voxel in the right PFC was chosen as previous fMRI studies report effects of anxiety in the right PFC [13, 14]. A medial position was chosen as lateral voxels can be harder to place due to tissue boundaries. The voxel was positioned manually by reference to an axial T1- weighted gradient echo image (Figure 3B). Spectra were acquired using SPin ECho full Intensity-Acquired Localised spectroscopy (SPECIAL; [189]) ^{1}H-MRS sequence with water suppression (TR 3000 ms, TE 8.5 ms, Phase cycle Auto, 192 averages from the right PFC voxel) in each participant [190]. Water unsuppressed spectra

(16 averages) were also acquired. Six outer volume suppression slabs were applied (one on each side at 5mm from the edge of the cubic voxel) to suppress signals originating from outside the volume of interest and to minimise motion-related image-selected in vivo spectroscopy subtraction artifacts. Spectra were analysed using LCModel 6.3-1L with the basis set consisting of 19 simulated basis spectra; alanine, ascorbate, aspartate, creatine (Cr), GABA, glucose, glutamine (Gln), Glu, glycine, glutathione, glycerophosphocholine, phosphocholine, lactate, myo-inositol (mI), N-acetylaspartate (NAA), N-acetylaspartateglutamate, phosphorylethanolamine, scyllo-inositol & taurine.

The basis set was simulated using FID-A [191], for TE = 8.5 ms, magnetic field strength = 3 T and assuming ideal RF pulses. Spectra with Cramer-Rao lower bounds (CRLB) > 20% as reported by LCModel were excluded. In addition to metabolite levels, line widths and signal-to-noise ratios were estimated by LCModel. All spectra had a Line Width < 8 Hz and an SNR > 40 [190].

Metabolite levels have been shown to depend on the amount of cerebral spinal fluid (CSF), gray (GMV) and white matter (WMV) within the voxel [192], and inter-individual differences in cortical gray matter [193]. Correlations between PFC Glu and GMV and WMV were calculated. To account for potential confounds the T1-weighted anatomical images were used to estimate the gray and white matter content of the right PFC voxel in which the ^{1}H-MRS measures were performed using GABA Analysis Toolkit (Gannet 2.0, http://gabamrs.blogspot.co.uk/) adapted to work with

Siemens SPECIAL data. The segmentation was performed using "new segment" in SPM 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). CSF, GMV and WMV were then accounted for in the expression of Glu and GABA levels using LCModel [194, 195]; corrected metabolite levels are referred to as *Glu Corr* and *GABA Corr* using the formula *Glu Corr = (Glu * (43300 * GMV + 35880 * WMV + 55556 * CSF)) / (35880 * (1 - CSF))* and *GABA Corr = (GABA * (43300 * GMV + 35880 * WMV + 55556 * CSF)) / (35880 * (1 - CSF)).*

Additionally, because previous studies investigating the relationship between Glu and BOLD signal during cognitive control have used metabolite ratios relative to the synchronously-acquired Cr signal [87, 196] results based on Glu/Cr are reported in Appendix 3. Differences between LTA and HTA groups in right mPFC metabolite levels, as well as SNR, Line Width and CRLB were established using independent sample t-tests. Additionally, the BF_{10} for each comparison was calculated to assess the likelihood of the model relative to the null hypothesis.

2.2.7. *fMRI Data Analysis*

Functional MRI data were analysed using the Statistical Parametric Mapping software package (SPM12, Welcome Department of Cognitive Neurology, London, UK, www.fil.ion.ucls.ac.uk/spm/spm12). The anatomical and Echo Planer images (EPI) were reoriented manually based on the anterior commissure - posterior commissure axis. The images were corrected for slice timing. Motion correction was performed for functional images using six movement parameters to reduce motion artefacts.

Volumes were co-registered to the high-resolution T1-weighted image and normalised into the Montreal Neurological Institute (MNI) template using parameters generated by unified segmentation of the T1-weighted structural image. The transformed data were smoothed using an 8 mm full width at half maximum (FWHM) isotropic Gaussian kernel. A high-pass filter with a cut-off of 128 s was applied to reduce low-frequency noise.

A fixed effects general linear model (GLM) was used to model data from the Stroop task at the 1st level based on event-related Congruent and Incongruent colour-word trials. The number of error trials were modelled as regressors of no interest and Fixation cross trials were modelled implicitly. The six motion correction parameters were included as regressors of no interest in 1st level models. Contrast images were created for each participant at the 1st level to examine the main effect of condition (Congruent vs. Incongruent). The contrast Incongruent > Congruent was specified for each 1st level model to establish the effect of interference on whole brain activity at the single subject level.

These 1st level contrasts were then entered into a second-level ANCOVA to examine the main effect of task (Incongruent > Congruent trials). To assess the effect of trait anxiety on DLPFC activation the 1st level contrast images were entered into a regression model in SPM v12 as power was insufficient to detect small to medium effects using an independent samples t-test.

These 1st level contrasts were entered into a second-level ANCOVA with each participants trait anxiety group (LTA vs. HTA) and PFC Glu Corr

levels to examine task-related activation during incongruent trials (Incongruent > Congruent), the effect of trait anxiety group on task-related activation and the interaction effect for group x Glu Corr levels. Furthermore, each participant's mean ER was included as a covariate of no interest to control for the effects of task performance on brain activation as these different between LTA and HTA groups. As the effect of Group on estimated IQ scores was non-significant estimated IQ was not included as a covariate in ANCOVA.

Because of the a priori hypothesis that trait anxiety would specifically be associated with increased activity in DLPFC regions during a task requiring cognitive control an ROI approach (x, y, z = +/-34, 36, 24, small volume correction (SMV) sphere = 12mm) was used. The DLPFC ROI was based on previous reviews of fMRI tasks that manipulate cognitive control [197, 198] and a previous study which reports a positive correlation between trait anxiety and DLPFC activity during a high load condition [6]. As effects of anxiety have been reported in left [6], right [13, 14, 199] and bilateral DLPFC activity [15, 65] a bilateral DLPFC ROI was chosen. Exploratory full brain analyses are reported in Appendix 3. For all analyses ER were included as a covariate of no interest. Significance results are reported at a threshold of $p<.05$ (FWE-peak-level). To represent results graphically parameter estimates of activation were extracted from the peak voxel in analyses. No secondary analyses were performed on the extracted values [200, 201]. Plotting served the purpose of disentangling the effect revealed in the GLM.

2.3. **Results**

2.3.1. *Trait Anxiety Groups*

A median-split based on STAI trait scores (median = 42) was used to establish LTA and HTA groups. LTA and HTA groups differed significantly on STAI trait and state anxiety scores but not in age, or estimated IQ scores. There were no significant group differences between groups in alcohol consumption or cannabis use (see Table 2).

Table 2

STAI scores, age, estimates IQ and substance use between groups.

	LTA (n = 19)	**HTA (n = 20)**	**Analysis**
STAI trait	33.05 (5.05)	49.20 (9.33)	$t(37) = -6.67$, $p < .001$
STAI state	27.79 (5.41)	38.79 (10.76)	$t(37) = -4.83$, $p < .001$
Age (years)	22.31 (5.09)	21.80 (4.25)	$t(37) = .34$, $p = .73$
Estimated IQ	109.00 (9.91)	109.30 (10.80)	$t(37) = .01$, $p = .93$
Cannabis use (Moderate)	2	0	U = 155, $p = .27$
Alcohol use (Regular)	3	1	U = 183, $p = .78$

2.3.2. *Task Performance*

2.3.2.1.Task Performance between Trait Anxiety Group

Error Rates: Participants' ER and RT during the Stroop task are shown in Figure 2. ANOVA revealed a significant effect of condition for ER ($F(1, 37) = 24.89$, $p < .001$, $\eta_{part}^2 = .40$) with a greater ER during incongruent trials across all participants. There was also a significant effect of trait

anxiety group on ER (F(1, 37) = 4.63, p = .038, η_{part}^2 = .11) and significant group x task condition interaction effect (F(1, 37) = 7.59, p = .009, η_{part}^2 = .17) revealing that ER were greater in the incongruent condition for the HTA group.

Reaction Times: The main effect of condition on RT was non-significant (F(1,37) = 1.84, p = .183, η_{part}^2 = .05); however, there was a significant effect of trait anxiety group on RT (F(1, 37) = 4.54, p = .040, η_{part}^2 = .11). Across the task, the HTA group were slower than the LTA group. The group x task condition interaction was non- significant (F(1, 37) = 0.13, p = .717, η_{part}^2 < .01). The relative likelihood of this model compared to the null hypothesis is BF_{10} = 0.29.

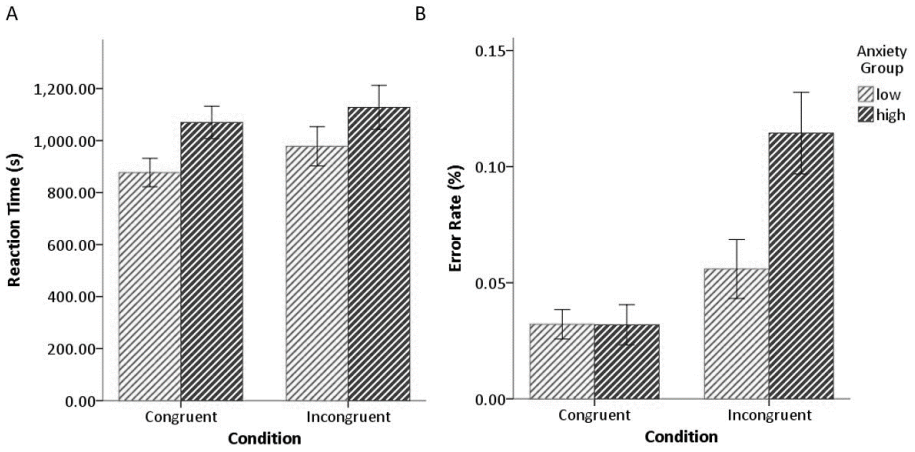

Figure 2. Reaction time and error rate data for Stroop task. (A) Mean reaction time in milliseconds (ms) and (B) error rate % errors by trait anxiety group and task condition. Error bars show the standard error of the mean.

2.3.3. *fMRI: Stroop Effect*

Compared to Congruent trials, Incongruent trials were associated with activation in the bilateral Medial Superior Frontal Gyrus and ACC, the

bilateral Precentral Gyrus extending to the right Middle Frontal, and in the left Middle Frontal and Inferior Gyrus and Putamen (see Figure 3 and Table 3). There was no significant activation in the opposite contrast (Congruent > Incongruent trials) at a FWE corrected level of p <.05.

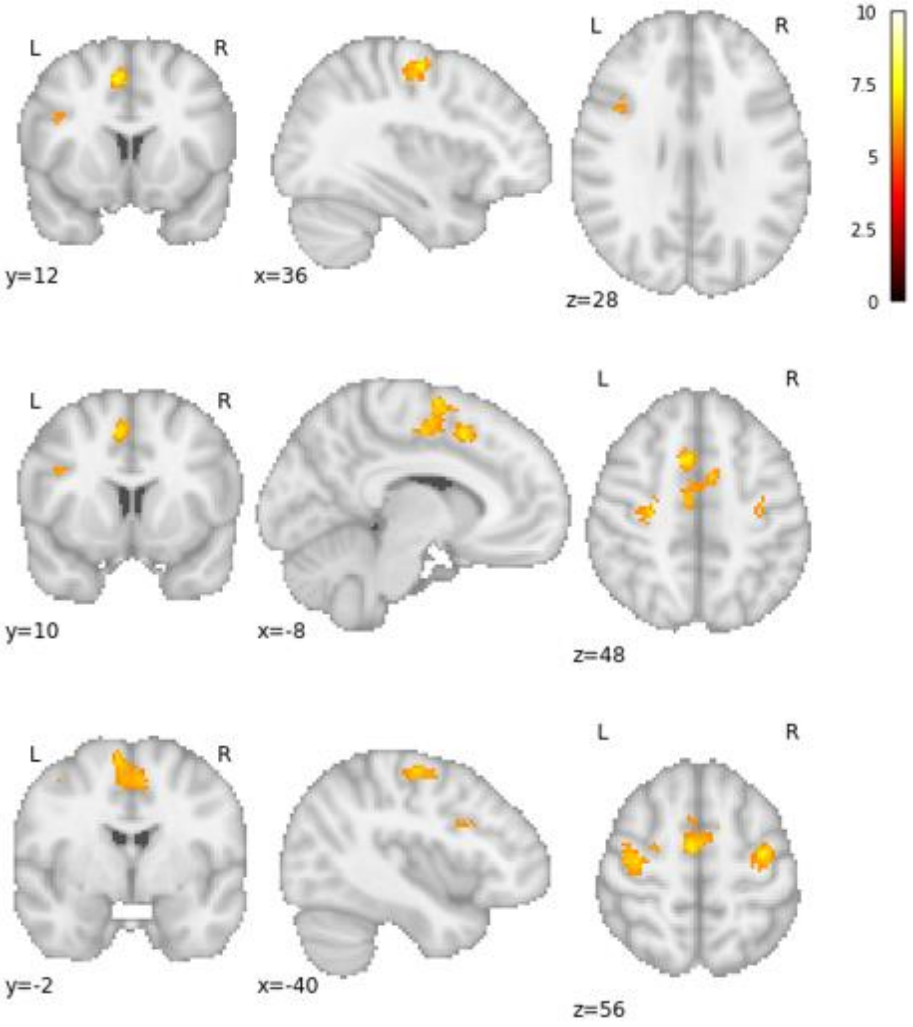

Figure 3. (A) Statistical Parametric Maps in axial, coronal and sagittal sections showing the main effect of the Stroop task (incongruent > congruent) in cortical regions. Results displayed at p < .05 FWE peak corrected.

Table 3

Regions and MNI coordinates for activations during Incongruent > Congruent Stroop Trials (p FWE peak < .05).

				MNI coordinates (mm)		
Cluster	**Hemisphere**	**$P_{FWE\ (Peak\text{-}level)}$**	**Z**	x	y	z
ACC/Superior Frontal Gyrus	R	<.001	5.87	-8	12	48
		<.001	5.77	-6	-8	52
		0.001	5.55	-8	-4	66
Precentral Gyrus	R	<.001	5.70	36	-12	56
		0.024	4.91	32	-20	50
Precentral Gyrus	L	0.001	5.55	-36	-16	56
		0.001	5.53	-28	-18	48
		0.006	5.23	-26	-10	52
ACC	R	0.008	5.17	16	16	34
		0.022	4.93	10	16	40
Inferior Frontal Gyrus/ Precentral Gyrus/ Middle Frontal Gyrus	L	0.021	4.95	-40	10	28
Precentral Gyrus/ Middle Frontal Gyrus	L	0.025	4.90	-38	0	40
Insula	L	0.026	4.90	-42	22	0
Posterior Supramarginal Gyrus	L	0.035	4.83	-54	-46	22
Precentral Gyrus/ Middle Frontal Gyrus	L	0.041	4.79	-36	-2	44
Middle Frontal Gyrus/ Inferior Frontal Gyrus	L	0.042	4.78	-38	18	28
Putamen	L	0.049	4.74	-24	0	14

2.3.4. *Effect of Trait Anxiety on DLPFC Activity during Incongruent Trials*

The effect of trait anxiety (STAI trait scores) on DLPFC activation was non-significant in bilateral DLPFC ROI during Incongruent > Congruent trials.

2.3.5. *^{1}H- MRS: Glu Corr and DLPFC Activation*

PFC Glu Corr metabolite levels and spectra quality control data for LTA and HTA groups are reported in Table 4. All other metabolite levels are reported in Table 3. Differences between LTA and HTA groups for right PFC Glu Corr were non-significant (relative likelihood of this model compared to the null hypothesis $BF_{10} = 0.64$).

Table 4

Means, Standard deviations and statistical analysis/Bayes Factors for ^{1}H-MRS quality control measures, right medial PFC Glu and GABA levels (Corr & /C) by LTA and HTA groups. Metabolite levels are represented in arbitrary units.

PFC Metabolite Levels				**Analysis (LTA vs. HTA)**	
	LTA	**HTA**	**Total**	t-test result	BF_{10}
Creatine	6.57 (.44)	6.37 (.59)	6.47 (.52)	t(37) = 1.17, p = .249	0.54
GABA	1.73 (.22)	.1.83 (.33)	1.78 (.28)	t(37) = 1.06, p = .296	0.49
GABA Corr	1.97 (.27)	2.14 (.40)	2.06 (.35)	t(37) = -1.62, p = .113	0.87
GABA/Cr	.26 (.03)	.29 (.06)	.28 (.05)	t(37) = -1.65, p = .107	0.90
Gln	.19 (.05)	.21 (.07)	.20 (.06)	t(37) = -.93 p =.360	0.44
Glu	6.54 (.46)	6.64 (.68)	6.59 (.58)	t(37) = -.52 p =.609	0.35
mI	5.73 (.50)	5.62 (.44)	5.67 (.47)	t(37) =.70 p =.489	0.38
NAA	8.50 (.44)	8.29(.81)	8.39 (.66)	t(37) = 1.02 p = .315	0.47
Glu Corr	7.41 (.58)	7.80 (1.10)	7.61 (.90)	t(37) = -1.36, p = .183	0.64
Glu/Cr	1.00 (.06)	1.05 (.08)	1.02 (.08)	t(37) = -1.99, p = .054, $\eta part^2$ = .097	1.44
SNR	60.00 (4.77)	60.85 (7.01)	60.44 (5.96)	t(37) = -.44, p = .662	0.34
Line Width in Hz	3.53 (.79)	4.26 (1.30)	3.90 (1.13)	t(31.67) = -2.128, p = .041, $\eta part^2$ = .107	1.71
Glu CRLB	4.05 (.62)	3.85 (.67)	3.95 (.65)	t(37) = .98, p = .335	0.46

There was a significant interaction between PFC Glu Corr levels and trait anxiety group in the left DLPFC ROI (x, y, z = -26, 30, 18, Z = 3.60; $P_{FWE\ (Peak\text{-}level)}$ = .044) (*Figure 4*C). The scatter plot in *Figure 4*A shows that during incongruent trials (Incongruent > Congruent) the LTA group showed a positive association between PFC Glu Corr levels and brain activity in the left Middle Frontal Gyrus.

In the HTA group, during incongruent trials, PFC Glu Corr levels were not associated with activation in the DLPFC ROI. This interaction effect was not accounted for by task performance (ER).

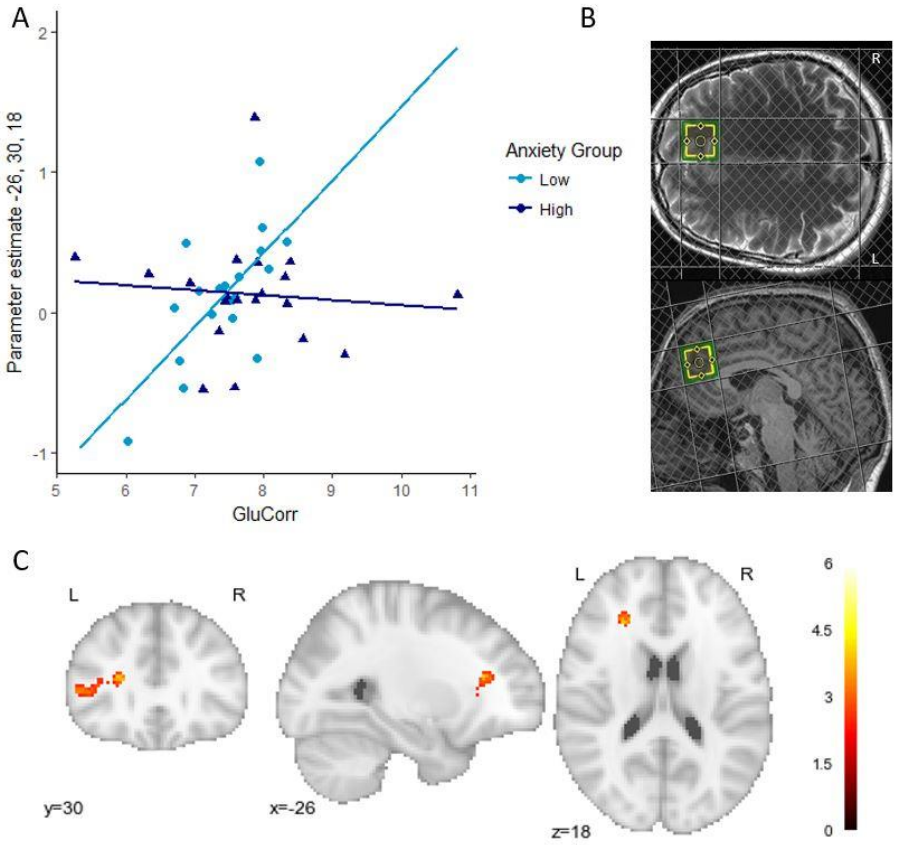

Figure 4. (A) Scatter plot and line of best fit showing individual contrast parameter estimates by right PFC Glu Corr levels (arb. unit) by trait anxiety group. (B) Positioning of the voxel for right medial PFC voxel for ^{1}H-MRS acquisition. (C) Statistical Parametric Map showing brain activations for trait anxiety Group x PFC Glu Corr interaction during incongruent trials at P =.05 FWE corrected threshold. Results displayed at p > .005 uncorrected for illustrative purposes.

2.4. **Discussion**

The aim of this first study was to examine the relationship between trait anxiety, DLPFC activation during a cognitive control task, and PFC Glu levels. Overall, participants performed the Stroop task with a high level of accuracy. As expected, during the Stroop task, ERs were greater during incongruent trials although unusually, RTs did not differ significantly between congruent and incongruent conditions. It is unclear why this RT pattern was observed but may be due to a speed accuracy trade-off or trial/task pacing [202]. However, relative to the LTA group, the HTA group had greater ER during incongruent trials and were generally slower across the task. Reduced task performance (i.e. increased ER and RT) in the HTA group is consistent with the prediction that high levels of trait anxiety reduce *performance effectiveness* [7]. Reduced *performance effectiveness* during the incongruent trial condition of the Stroop task has been reported previously in anxious individuals [13, 203] and may be related to the high cognitive control requirements of the task.

During the Stroop task, fMRI data showed that incongruent (> congruent) trials were associated with activity in the ACC and Medial Superior Frontal Gyrus, the bilateral Precentral Gyrus, right Middle Frontal Gyrus and left Middle and Inferior Frontal Gyri (as well as smaller activations in a number of subcortical regions). This finding is broadly consistent with previous fMRI studies/meta-analyses reporting functional activation during the Stroop task (e.g., [13, 204-206]). It is assumed that incongruent trials increase activity in ACC, SMA, and DLPFC regions due to the increased need for cognitive control.

In individuals with HTA, increased DLPFC activation without improved task *performance effectiveness* has been interpreted as reduced *processing efficiency* [13-15]. However, contrary to some previous fMRI findings, trait anxiety was not significantly associated with increased activation in the DLPFC during incongruent trials. Nevertheless, in the present study, the HTA group did demonstrate reduced *performance effectiveness* relative to the LTA group, suggesting that their DLPFC activation during incongruent trials may have been insufficient to perform the task effectively.

It has been reported previously that cortical Glu levels can predict anxiety levels [96] and that pharmacologically induced anxiety increases cortical Glu levels [180]. Examining this ^{1}H-MRS data, however, there were no significant differences in PFC Glu levels between LTA and HTA groups. This may be due to the ^{1}H-MRS voxel placement, in the medial PFC, which differed from the ACC voxel placement used in these previous studies [20, 21]. It was then examined how trait anxiety influenced the relationship between PFC Glu levels and DLPFC activation during cognitive control. There was a significant interaction between PFC Glu levels, trait anxiety and left DLPFC activation during incongruent task trials. This effect was driven by a positive association between PFC Glu levels and DLPFC activation in the LTA group, while PFC Glu and DLPFC activation were unrelated in HTA participants. This finding suggests a role for resting-state PFC Glu in DLPFC activation and is in line with previous studies by Falkenberg and colleagues [87] and Duncan and colleagues [196] that report resting-state Glu levels significantly

influence how the brain implements cognitive control. Although speculative, resting-state PFC Glu may facilitate efficient processing during cognitive control through a higher capacity for energy turnover [207] and/or NMDAR function [97] that increase DLPFC activity in line with task demands.

It should be made clear, however, that the relationship between resting-state Glu concentrations and neural energy metabolism in humans is not fully understood [208, 209]. Thus, in the LTA group it is possible that such a positive relationship between excitatory neurotransmission and task-related activation in the DLPFC facilitates an effective and/or efficient neural processing mechanism when cognitive control is required. On the other hand, in the HTA group, no association between resting-state Glu levels and DLPFC activity was observed. This could be due to effects of trait anxiety on NMDAR function. Anxiety and neuroticism (a personality construct closely linked to trait anxiety) have been shown to affect NMDAR function [210, 211] and differences in NMDAR function can effect task-related interactions between default mode and FPN regions [97, 212]. The absence of this relationship between resting-state PFC Glu levels and DLPFC activity in the HTA group may result in ineffective task performance; consistent with the predictions of ACT [7]. Together, these findings provide new insight into how a normally distributed personality dimension such as trait anxiety can affect the relationship between excitatory neurotransmission and activation in neural regions that support cognitive control. Future work could investigate if modulation of

excitatory neurotransmission can ameliorate anxiety-related effects on cognition.

2.4.1. *Limitations*

First, power calculations suggest that whilst the study was sufficiently powered to detect medium to large effect sizes, the sample may have been too small to detect small differences (i.e. small effect size) in ^{1}H-MRS metabolite concentration between LTA and HTA groups. Thus, the null findings reported here, i.e. no differences between groups for PFC Glu levels and other metabolites, need to be interpreted with some caution and future studies aiming to examine the effect of trait anxiety on PFC Glu would need to recruit larger samples. It should also be noted that four of the 39 study participants were left-handed and laterality may affect Stroop task performance [213].

Second, ^{1}H-MRS-fMRI analyses did not show any interaction effects within the right medial PFC voxel itself. Similar findings have been reported previously [87, 196], where no relationship between Glu and BOLD signal was seen in the measured region. This points to a more global effect of Glu on BOLD response, exerting 'long-range' influence on other regions via glutamatergic projection [87]. Notably this study relies on resting-state Glu measurements rather than examining changes in these metabolite levels as a result of task demands. Though the use of resting-state ^{1}H-MRS is common practice, PFC Glu levels differ between rest and task and reflect changes in other metabolic measures and cognitive demands [214]. Thus, future work should measure task-related differences

in Glu levels to obtain a more accurate insight of the neural basis of cognitive processes [85].

Third, the concept of *processing efficiency/inefficiency* that is central to ACT does not tell us about the precise neural mechanisms that underlie the different patterns of brain activation in people with high levels of anxiety. For example, differences in intensity and timing of neural signalling (i.e. temporal dynamics) as well as resting cerebral blood flow and metabolism would be likely to affect activation in fMRI experiments [215]. However, it was shown here that excitatory neurotransmission can modulate task-related activation in the PFC and that this modulation effect is perturbed in people with HTA. Finally, there is emerging evidence that cognitive deficits in people with HTA/anxiety disorders are partly due to functional network imbalances (see [36]). Future work should examine how network interactions (i.e. FPN and DMN) are modulated by excitatory/inhibitory neurotransmission and how these interactions are affected by anxiety.

2.4.2. *Conclusions*

We have demonstrated that individual differences in trait anxiety affect the relationship between PFC Glu levels and DLPFC activation during cognitive control. This may contribute to ineffective task processing when cognitive control is required. These results need to be replicated in larger samples and more work is needed in order to examine how task-related excitatory neurotransmission during cognitive control is affected by trait anxiety.

3. Methodology for rt-fMRI-nf Protocol

3.1. Design

A mixed between- and within-subjects experimental fMRI design was employed to test if functional connectivity-based rt-fMRI-nf could be used to modulate brain activity/connectivity, attentional control and anxiety levels in people with HTA. Participants were recruited via online screening and subsequent phone interview and were pseudo randomly assigned to an experimental (EG) and control group (CG). The EG received rt-fMRI-nf based on the functional connectivity between left DLPFC and the bilateral ACC ROIs, while the CG received sham feedback based on brain activation of a single articipant in the experimental group (yolked feedback, e.g., [133]). Participants in both groups (N = 32) underwent two rt-fMRI-nf sessions on separate days that were scheduled one week apart (pre- and post-) i.e. two separate visits to the MRI scanner at the Combined Universities Brain Imaging Centre (CUBIC). For three participants, the interval between sessions was two weeks due to technical problems with the MRI scanner. Assessment measures (i.e. psychometric, cognitive task and MRI data) were collected at both pre- and post- rt-fMRI-nf training time points. (see *Figure 5*).

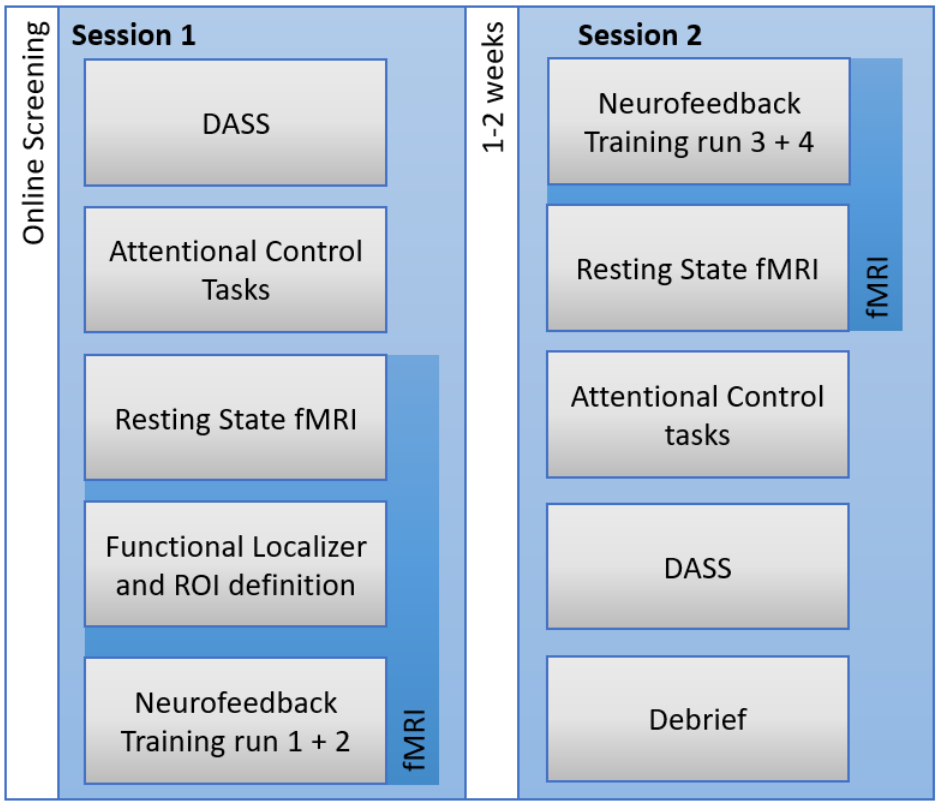

Figure 5. Experimental Design for rt-fMRI-nf protocol. DASS: Depression, Anxiety and Stress Scale.

3.2.**Study Sample**

The State Trait Anxiety Inventory (STAI; [4]) has largely shaped the current definition of trait anxiety, as a personality dimension characterised by intrusive thoughts, worry and difficulty disengaging with irrelevant information. Moreover, it is the most established measure of trait anxiety in psychology [2]. Previous research has used the STAI to characterise trait anxiety, identify and divide samples into subgroups of high, medium and low trait anxiety (e.g., [13, 216, 217]). For the purpose of this study only individuals with HTA were recruited using the STAI in recruitment.

3.2.1. *Recruitment*

Participants were recruited via online screening using Qualtrics (Provo, UT). The study was advertised at the University of Roehampton, Royal Holloway University of London, and to the general public. Between 12/06/2017 and 25/06/2018 603 participants completed the online screening battery comprising the STAI to assess levels of trait anxiety and a number of filter questions regarding eligibility for the study (e.g., local availability and no history of psychiatric or neurological conditions). Participants scoring in the upper quartile (above a percent rank of 75%) of the trait anxiety scale on the STAI (defined based on the sample distribution of the first 100 respondents at a score of 49 or above) were then contacted by phone and underwent a brief interview to establish eligibility for the study. Inclusion criteria were as follows: age between 18 to 35 years, no prior neurological illness, were not using medication for anxiety or depression, no evidence of alcohol or drug dependence and no contraindications for exposure to a magnetic field (e.g., magnetic implants). Participants meeting inclusion criteria were invited to participate in the rt-fMRI-nf experiment.

For all respondents (N = 603), the mean score for STAI state anxiety was 36.70 (SD = 11.04, range 20 - 71), and the mean score in STAI trait anxiety was 42.14 (SD = 11.79, range 20 - 77). The median STAI trait anxiety score was 40. This is comparable with previously reported norms in healthy samples (e.g., [4, 6]).

3.2.2. *Ethics and Informed Consent*

Ethical approval was granted by the University of Roehampton Ethics Committee (Appendix 2.2.). The study complied with the BPN Code of Ethics and Conduct (2009) and the Code of Human Research Ethics (2014). All participants gave written informed consent prior to taking part in the study. Participants were explicitly informed that they may be in the control group, while the experimenter would treat everyone as they would a participant in the experimental group (i.e. all groups received identical instructions throughout the study). All participants were fully debriefed upon completion of the study.

3.2.3. *Data Protection and Confidentiality*

To ensure confidentiality participants were assigned a unique ID code and only members of the research team had access to this information. The processed MRI data was likewise stored with an ID code. This ID code was used to link MRI data with participant's demographics, questionnaire and behavioural data. The participant's initials and the date of scanning were embedded in the imaging data files for the MRI Unit at Royal Holloway to be able to contact the person's GP in the case anomalies are detected during the scanning; the data will however be strictly confidential.

3.2.4. *Participants*

A total of 32 HTA participants were recruited for the rt-fMRI-nf experiment, two of whom did not complete the full study protocol due to claustrophobia and a technical issue, consequently full data in 30 participants were available. Participants (22 female, 8 male) ranged from

18-33 years of age (M = 21.00 years, SD = 3.67) and had a mean estimated IQ of 109.24 (SD = 5.09, range 98.06 - 119.57) as measured by the National Adult Reading Test [218, 219]. There were 28 right handed and 2 left handed participants as assessed by self-report. Participants spent an average of 15.20 years (SD = 1.77) in full time education (beginning with primary education). Alcohol consumption and recreational cannabis use were assessed for all participants using a categorical scale (ranging from no-use to regular use). Most participants indicated that they used alcohol on a moderate basis and that they used cannabis never or only experimentally (see *Table 5*). There were no significant differences between groups in alcohol consumption (U = 106, p = .795) or cannabis use (U = 101.5, p = .589).

Table 5

Frequency of alcohol and cannabis consumption across participants.

	No use	Experimental use	Occasional use	Regular moderate use	Severe use
Tobacco	26	0	0	3	1
Alcohol	5	0	20	5	0
Cannabis	21	5	4	0	0

Participants in EG and CG did not differ on age, t(28) = -0.79, p = .435 (EG M = 21.53, SD = 4.36; CG M = 20.47, SD = 2.88), estimated IQ scores; t(28) = -0.03 p = .97 (EG M = 21.53, SD = 4.36; CG M = 20.47, SD = 2.88), years spent in full time education; t(28) = -0.61, p = .55 (EG M = 21.53, SD = 4.36; CG M = 20.47, SD = 2.88) and gender distribution (EG N_{female} = 11, CG N_{female} = 11). The groups differed in handedness, as both left handed participants were in the EG.

For the 30 participants who completed the rt-fMRI-nf training protocol the mean STAI trait anxiety scores was 56.47 (SD = 5.84, range 49 - 71) and the mean STAI state anxiety score was 45.70 (SD = 9.91, range 28 - 66) at the time of recruitment. Participants pseudo-randomly assigned to EG and CG did not differ on STAI trait anxiety scores $t(28) = 1.07$, $p = .296$ (EG M = 55.33, SD = 5.19; CG M = 57.60, SD = 6.40) nor on STAI state anxiety $t(28) = 0.34$, $p = .733$ (EG M = 45.07, SD = 9.32; CG M = 46.33, SD = 10.75) at the time of the online screening. The STAI trait anxiety scores in both EG and CG were above the 70^{th} percentile of the distribution based on published norms [4].

3.3. Power Calculation

To test if analyses were sufficiently powered, G*Power was used. Power calculations suggest that, with independent group sizes of n = 15 (EG & CG), the experiment would have sufficient power to detect a significant group difference (using a repeated measures ANOVA) for effect sizes > .6 (medium to large), sufficient power to detect differences within groups over time for effect sizes of > .34 (small to medium) and sufficient power to detect a group x time interaction for effect sizes of > .34 (small to medium). Thus, the sample size is insufficient to detect small effect sizes within groups differences and for interaction terms and insufficient to detect small to medium effect sizes for between group differences.

3.4. **Neuroimaging**

3.4.1. *Image Acquisition*

3.4.1.1. Structural Scan

All MRI scans were acquired on a 3T Siemens Magnetom TIM Trio scanner using a 32-channel head coil. Structural T1 weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MP RAGE) images were acquired with a spatial resolution of 1 mm × 1 mm × 1 mm, in plane resolution of 256 × 256 × 176 slices and scanning time of approximately 5 minutes.

3.4.1.2. Functional Localiser and Neurofeedback Scans

A multiband frequency protocol was used for both the functional localiser and all rt-fMRI-nf scans. TR/TE/flip angle = 1 s/33 ms/70°, field of view 192 mm × 192 mm and slice thickness of 3 mm giving a voxel size of 3 mm × 3 mm × 3 mm and whole brain coverage of 48 interleaved slices. 360 volumes were acquired in the functional localiser with a scanning time of 6 minutes. 420 volumes were acquired in each of the two rt-fMRI-nf runs, each rt-fMRI-nf run had a scanning time of 7 minutes.

3.4.2. *Tasks during fMRI Scanning*

3.4.2.1. Functional Localiser

Participants performed a modified colour-word Stroop task [184] adapted for fMRI and used previously [187]. This task served to functionally localise the bilateral ACC and left DLPFC and to calculate individual connectivity parameters based on these regions to scale rt-fMRI-nf to the

individual range of functional connectivity in each participant. The functional localiser task was customised using Python and presented in PsychoPy (Pierce 2007).

The Stroop task was presented using a block design with two conditions, Rest and Task. Task blocks consisted of only incongruent trials to achieve maximum power to detect brain regions activated during attentional control. Blocks lasted for 30 seconds and there was a total of six blocks per condition. At the beginning of each block, instructions were presented visually (200 ms) instructing participants to either "REST" or "ATTEND". During Rest blocks participants were instructed to relax with their eyes open. During Task blocks participants responded with one of four fingers of their right hand to the font colour (Red, Blue, Green, & Yellow) of the word presented in the middle of the screen (Red, Blue, Green, & Yellow) for incongruent Stroop trials. Each trial took 5000 ms with an inter-stimulus interval of 3000 ms. The presentation time for each stimulus was 1000 ms. Participants were allowed 2000 ms from stimulus onset to respond (i.e. responses were registered from the onset of each stimulus trials). Participants were instructed to respond as quickly and as accurately as possible.

3.4.2.2.Neurofeedback Training

All participants underwent four rt-fMRI-nf runs; two runs in the first and two in the second session (scanner visit). Participants were instructed to '*try to move the gauge on the screen upwards*'. No specific examples of strategies were given [107], and participants were encouraged to change

strategy until they found a successful way to regulate their left DLPFC-bilateral ACC functional connectivity, as represented via the visual feedback interface. Participants were informed about the inherent delay in the visual feedback signal due to the hemodynamic delay and the way the signal was calculated. Participants were encouraged to maintain new strategies for longer periods of time to establish their full effect.

Participants were informed that they may be in the CG and would thus receive sham-feedback. The CG received identical instructions to the EG, while the feedback display they viewed corresponded to activation from a previously tested participant in the EG (yolked feedback, e.g., [133]). This was to achieve the same visual displays and sense of self-efficacy in the CG and the EG.

Each rt-fMRI-nf run consisted of six Rest (25s) and six Regulate blocks (45s). An example of the display during the rt-fMRI-nf training is shown in **Error! Reference source not found.**, during regulate blocks the number of wavy lines would vary from none to ten, depending on the sliding windowed (20 s) partial correlation between left DLPFC and bilateral ACC activation, while accounting for signal from the noise ROI. A greater number of wavy lines indicated an increased partial correlation coefficient between these regions. The feedback display was updated with every TR (1 s), (i.e. continuous feedback was given). The ROIs were defined using a localiser protocol described above and the feedback was

scaled to the individuals' minimum and maximum functional connectivity during a localiser scan (Section **Error! Reference source not found.**).

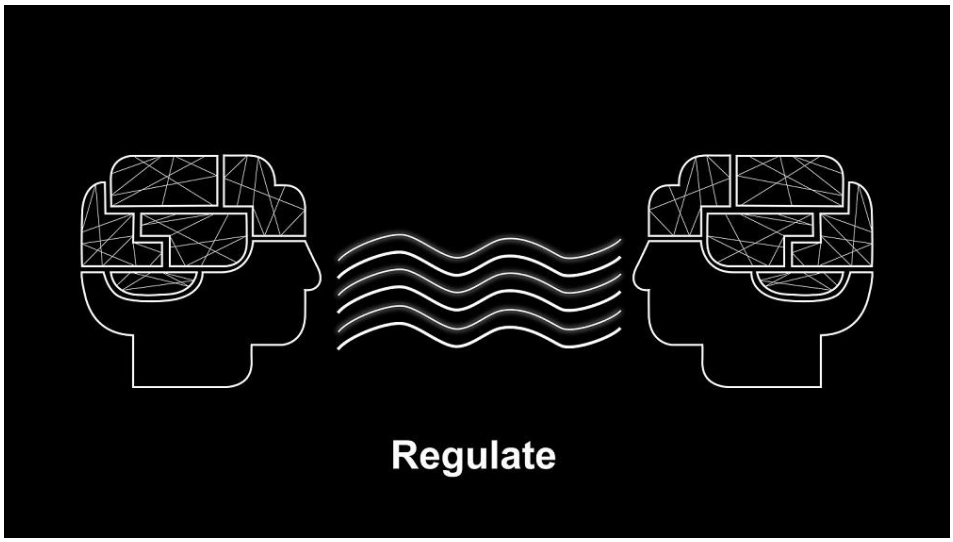

Figure 6. Example of Neurofeedback display used during rt-fMRI-nf training. Note, the number of wavy lines increased as participants achieved greater functional connectivity between DLPFC and ACC ROIs.

Participants were asked three questions in a short interview after both neurofeedback scans to explore their experience of trying to regulate their brain connectivity. They were asked which strategies they used to regulate their brain, whether they thought these led to successful up-regulation of the signal, and which strategy was most successful. Their answers were recorded as notes by the researcher while preserving the participants' choice of words where possible. Participant's responses are reported in Appendix 4.

3.5. Online fMRI Analysis

Real-time online analysis of fMRI data was performed with Turbo-Brain Voyager (TBV), Version 3.2 (BrainInnovation B.V., Maastricht, The Netherlands) and custom scripts in python for functions where appropriate

software was not readily available (available in Appendix 5 and at Open Science Framework DOI 10.17605/OSF.IO/SYNEU). For both the functional localiser and neurofeedback data, the reconstructed DICOM images were directly transferred to an analysis computer that was securely networked with the MR computer. Pre-processing was performed on the transferred images using TBV. This included Gaussian spatial smoothing with a smoothing kernel of 4 mm FWHM and motion correction. The functional data was registered to the anatomical scan of the respective session.

3.5.1. *ROI Definition*

The purpose of the functional localiser scan was to identify the bilateral ACC and left DLPFC and use these ROIs to calculate individual connectivity parameters in participants to scale the rt-fMRI-nf signal. The pre-processed BOLD signal was analysed with a GLM contrasting Task over Rest blocks (Task > Rest) in TBV. Based on the resulting t-maps, combined with anatomical landmarks, regions of interest (ROIs) were drawn manually over the left DLPFC and bilateral ACC. Generally a threshold of t = 2.40 was applied for ROI definition. However, the threshold varied between participants depending on the extent of above-threshold activation in an attempt to control the number of voxels in each ROI between participants. Figure 7 shows an example of the ROI placement in an individual participant.

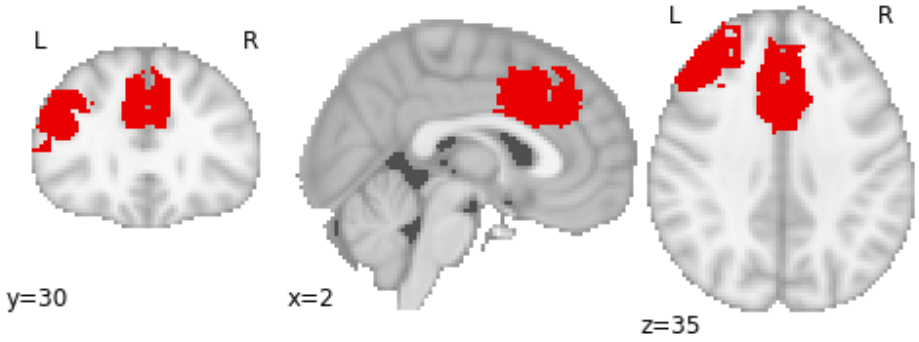

Figure 7. Combined binary ROI across all subjects in the bilateral ACC and left DLPFC registered to a standard MNI template.

In the EG the mean number of voxels in the left DLPFC ROI was 121.80 (SD = 39.90, range 23 - 198), the mean number of voxels in the ACC ROI was 108.80 (SD = 21.74, range 69 - 135). A third large rectangular ROI (nuisance), to account for general brain activation and global scanning effects, was drawn independently of the GLM covering a large area in the right Lateral Occipital Cortex, Superior Parietal Lobe and cerebral white matter. The mean number of voxels in this ROI was 324.47 (SD = 62.33, range 179 - 432).

In order to scale rt-fMRI-nf to individual participants, the time series from all three ROIs were extracted and a custom python script (Appendix 5.1.) was used to calculate partial correlations between DLPFC and ACC ROIs, while controlling for the third nuisance ROI. At the time of data collection, there was no readily available software to calculate dynamic functional connectivity between two ROIs while accounting for a third ROI. The custom script analysed the time series data from task blocks using a sliding window of 20 s and produced partial correlation values between the ROIs. Consequently the script removed correlation coefficients below 0 and

outliers (more than 2 SD from the mean). The minimum and maximum coefficients of the resulting values were used to scale rt-fMRI-nf. The mean minimum (ConnectivityBaseline) was a partial correlation of 0.17 (SD = 0.18, range 0.00 – 0.54) and the maximum (ConnectivityMax) was 0.81 (SD = 0.18, range 0.38 – 0.99).

3.5.2. *Calculation of Neurofeedback Signal*

The feedback participants received during rt-fMRI-nf training was based on the partial correlation between the left DLPFC ROI and the ACC ROI while accounting for a large nuisance ROI. All ROI were defined using the functional localiser and so were the values for ConnectivityBaseline and ConnectivityMax to scale rt-fMRI-nf signal. During the rt-fMRI-nf time course data was pre-processed in real time (Section 3.4.1.) and one value per ROI and TR was transferred to a networked computer running a custom python script (Appendix 5.2.). The python script read values as soon as they were available and calculated the partial correlation between values over a sliding window of 20 s. The following Formula I was implemented in the script to scale the derived correlation values to the individual range of functional connectivity values:

(I) $$Number\ of\ Lines\ = \frac{r_{DLPFCACC.noise} - ConnectivitBaseline}{ConnectivityMax - ConnectivityBaseline} \times 10$$

The resulting value was rounded to the next integer and values, while Number of Lines ≥ 10 resulted in the maximum feedback display of 10 and values ≤ 0 resulted in the minimum feedback display of 0 lines. With every new incoming set of time course (i.e. every TR), the script calculated a new value for Number of Lines and updated the visual feedback gauge.

The approach of combining established software (e.g., TBV) with custom additions to suit the needs for an experiment has been used in previous research (e.g., [104, 123]). The same ROIs were used in both neurofeedback sessions and were registered to the respective anatomical scan from the session.

4. Modulating DLPFC-ACC Functional Connectivity with rt-fMRI-nf

4.1. Introduction

Trait anxiety is the stable disposition to experience intrusive thoughts (i.e. worry) and to react to stressful situations with anxiety [3, 4]. HTA, similar to anxiety disorders and other psychiatric conditions, has been linked to altered activation and connectivity in the brain (e.g., [36, 41]). HTA has also been linked to impaired attentional control [17] and brain activation during attentional control tasks is altered in individuals with HTA [6, 13, 14, 34]. Impaired attentional control in HTA may be a contributing factor to anxiety.

ACT [7] provides a framework describing how anxiety can affect attentional control and exacerbate anxiety symptoms (See ref. [17] for review). Central to the model is the notion that anxiety and worry compete for limited processing resources in anxious individuals, occupying cognitive resources that would otherwise be allocated to attentional control [19, 220, 221]. Furthermore, the ability to inhibit negative thoughts and worry is reduced in people with HTA [7, 12]. Findings from fMRI studies are consistent with the predictions of ACT reporting that, whilst task or *performance effectiveness* is often maintained [7, 17, 21], HTA is associated with increased neural activity in regions important for attentional control, i.e. the DLPFC [13-15, 65, 82] and the ACC [71]. Increased activity in these regions without concomitant improvements in *performance effectiveness* is considered a form of *processing inefficiency*.

Moreover, functional connectivity studies (examining the temporal correlation between structurally distinct brain regions) report dysconnectivity between the DLPFC and the ACC in people with HTA and in people with high levels of worry - the main cognitive component of trait anxiety - during attentional control tasks [13, 34, 71]. The ACC is thought to be important for 'reactive' or 'compensatory' processes [37] that update the DLPFC when increased attentional control is required [79, 80]. Thus, DLPFC-ACC dysconnectivity could contribute to inefficient processing during attentional control tasks in people with HTA. DLPFC-ACC connectivity may also be indicative of coupling of wider attentional control networks in the brain [36, 83].

Based on the understanding of the role of impaired attentional control in people with HTA, a body of work has been conducted to modify anxiety by training attentional control performance [222-224]. However, these interventions have yielded mixed results [222, 223]. Typically, interventions to modify anxiety by training attentional control are based on increasing performance in cognitive tasks and are therefore inherently limited. Purely behavioural interventions cannot address the complex neural processes underlying impaired *processing efficiency* in individuals with HTA.

Rt-fMRI-nf is a recent development in neuroscience that enables participants to monitor and self-regulate their own brain activity in targeted brain regions (e.g., [133, 145, 147, 167, 225]). Recent work also shows the potential of rt-fMRI-nf to train connectivity between brain

regions (e.g., [104, 226, 227]). Neural changes induced by rt-fMRI-nf interventions have further been associated with improvements in clinical anxiety in people with spider phobia [167], PTSD [114, 141], and contamination anxiety [162]. Similarly, rt-fMRI-nf has been used to reduce non-clinical forms of anxiety by up-regulating brain activity in the amygdala [121] and by increasing functional connectivity between the amygdala and PFC [122].

Given the role of DLPFC-ACC functional connectivity during attentional control tasks [13, 71] and recent advances in rt-fMRI-nf studies to train brain activity and reduce anxiety, this study sought to examine the potential of connectivity-based rt-fMRI-nf, targeting DLPFC-ACC functional connectivity, in people with HTA. Specifically, it was hypothesised that veridical DLPFC-ACC functional connectivity-based rt-fMRI-nf training would modulate activity and increase functional connectivity in the DLPFC and ACC relative to sham. It was further hypothesised that increased activity and/or connectivity in DLPFC and ACC over the rt-fMRI-nf training period would be associated with reduced anxiety levels in the EG relative to the CG.

4.2. **Methods**

The study sample, experimental design and rt-fMRI-nf setup are described in detail in Chapter 3.

4.2.1. *Psychometric Assessment*

The Depression Anxiety Stress Scales (DASS; [228]) was used pre-rt-fMRI-nf training, and again post-rt-fMRI-nf training to assess short-term

changes in affective states. This 42-item scale measures affective states over the previous seven days and is therefore more sensitive to change in affect than the STAI trait measure [229]. The DASS is also designed to distinguish between feelings of depression, anxiety and stress allowing for a specific measure of changes in anxiety as opposed to depression and/or stress.

4.2.2. *Data Analysis*

Psychometric data were analysed using R 3.4.3 (R Core Team, 2017) and a significance threshold of $p < .05$ was applied throughout. fMRI data processing was conducted using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Significant results are reported at a threshold of $p < .05$ (Family Wise Error (FWE) -peak-level). A binarised grey matter mask based on the MNI structural atlas was used before thresholding to exclude voxels in white matter.

4.2.2.1.Psychometric Data

Questionnaire data were considered normally distributed after visual inspection. For each subscale of the DASS mixed-measures ANOVA was used with the between-subjects factor group (EG vs. CG) and time point (pre vs. post) as a within-subjects factor to establish the effect of rt-fMRI-nf training. Significant results were explored further with pairwise comparisons and reported at $p <.05$.

4.2.2.2.Functional Localiser Task

Functional localiser data were not available in one participant due to time constraints, hence the sample size in this task was n = 29 (EG = 15, CG = 14). A General Linear Model (GLM) was used to model data at the 1st level based on Task vs. Rest blocks. A Gamma convolution with a SD of 3 s and a mean lag of 6 s was applied and six motion correction parameters were included as regressors of no interest in all 1st level models. 1st level contrast images were created for each participant and then combined in a group Level analysis to evaluate the effect of Task > Rest.

4.2.2.3.Neurofeedback Training Runs

For rt-fMRI-nf runs 1 - 4, data were incomplete in one participant and were excluded from the analysis, hence the sample size was n = 29 (EG = 15, CG = 14). A General Linear Model (GLM) was used to model rt-fMRI-nf data at the 1st level using regressors for Regulate and Rest blocks. A Gamma convolution with a SD of 3 s and a mean lag of 6 s was applied and six motion correction parameters were included as regressors of no interest. 1st level contrast images were created for each rt-fMRI-nf run in each participant to examine the main effect of neurofeedback (Regulate > Rest). A 2nd level contrast contrasting rt-fMRI-nf run 1 with run 4 (run 4 > run 1) was then specified in each participant and submitted to a 3rd level independent t-test to establish the interaction between group (EG > CG) and rt-fMRI-nf run (run 4 > run 1). An ROI analysis with the left DLPFC and bilateral ACC ROI was performed to specifically test for changes in activation within the rt-fMRI-nf target regions.

In addition, a Psychophysiological Interaction Analysis (PPI) was conducted to examine rt-fMRI-nf-related changes in functional connectivity between ROIs using the left DLPFC ROI as a seed region. Additional 1st Level models were computed including regressors for the time series in the left DLPFC ROI in each participant and for the interaction of this time series with Regulation vs. Rest blocks. The same group level approach described above was used to test the interaction between group (EG > CG) and rt-fMRI-nf run (run 4 > run 1) in the PPI 1st Level contrasts. A ROI analysis with the ACC ROI was performed to specifically test for changes in connectivity between the left DLPFC seed region and the bilateral ACC.

To examine the association between changes in anxiety levels and activity and connectivity in left DLPFC and bilateral ACC ROIs during rt-fMRI-nf training in the EG, two regression analyses were performed. Differences in DASS anxiety scores between pre- and post- rt-fMRI-nf training were entered as a regressor into a model containing the contrast Regulate > Rest of all rt-fMRI-nf runs (runs 1 - 4) and secondly into a model containing the PPI estimates of all rt-fMRI-nf runs. ROI analyses were performed for both regressions. In the model with Regulate > Rest contrasts the left DLPFC and bilateral ACC ROI were used. For the model based on the PPI estimates the bilateral ACC ROI was used for ROI analysis.

4.3. **Results**

4.3.1. *Psychometric Results*

Comparing DASS Anxiety scores between EG and CG and between pre- and post-rt-fMRI-nf training showed that the effect of group ($F(1, 28) = 0.01$, $p = .938$), and time point ($F(1, 28) = 1.64$, $p = .211$) were non-significant. However, there was a significant interaction between group and time point ($F(1, 28) = 4.93$, $p = .035$, $\eta_{part}^2 = .150$) showing that at post-rt-fMRI-nf training the EG had reduced DASS Anxiety scores relative to pre- ($t(14) = 2.34$, $p = .035$, $d = 0.60$), an effect not seen in the

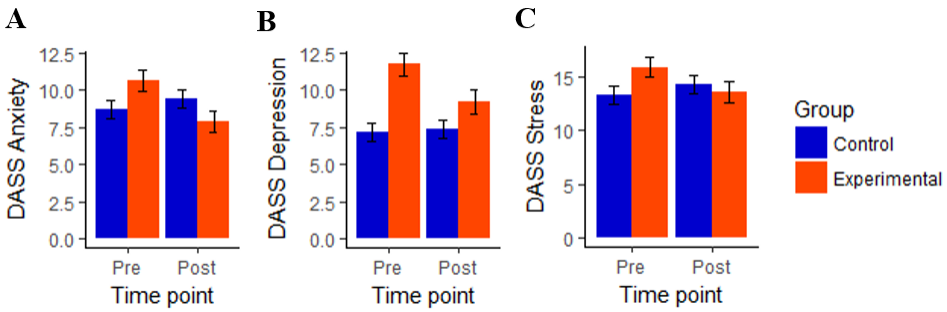

Figure 8. Mean DASS anxiety (A), depression (B) and stress (C) scores by Time Point and Group, error bars show 95% confidence interval.

CG ($t(14) = -0.71$, $p = .490$). Furthermore, this effect was specific to DASS Anxiety scores as comparison of pre- and post-rt-fMRI-nf training DASS Depression scores for group ($F(1, 28) = 2.80$, $p = .106$), time point ($F(1, 28) = 1.91$, $p = .178$), and interaction ($F(1, 28) = 2.61$, $p = .117$) and DASS Stress scores for group ($F(1, 28) = 0.11$, $p = .748$), time point ($F(1, 28) = 0.35$, $p = .559$), and interaction ($F(1, 28) = 2.33$, $p = .138$)) were non-significant (Figure 8).

4.3.2. *Functional Localiser Task*

Whole brain analysis of fMRI data showed that, during the functional localiser task (incongruent Stroop trials > Rest), activation was seen in the bilateral ACC (peak x/y/z = 6/18/32, Z = 9.78) and in the left (peak left x/y/z = -38/42/16, Z = 5.76;) and right (peak right x/y/z = 36/50/28, Z = 6.91) Middle Frontal Gyrus. Activation in further cortical, subcortical and cerebellar regions was also seen (see Table *6*).

Table 6

Regions associated with the task during the functional localiser.

Incongruent Stroop Trials > Rest	**Z-Value**	**MNI coordinates (mm)** x	y	z	
ACC	9.78	6	18	32	R/L
Superior Parietal Lobe	8.93	-40	-44	50	L
Insular Cortex	8.92	34	16	0	R/L
	7.42	-32	16	0	
Supramarginal Gyrus/					R
Postcentral Gyrus	8.13	40	-40	38	
	5.7	58	-20	24	
Cerebellum	7.33	22	-52	-30	R/L
	6.45	-36	-64	-30	
	5.73	18	-60	-54	
	4.91	8	-74	-46	
	4.81	-36	-58	-56	
	4.77	-40	-38	-40	
Frontal Pole/ Middle					R/L
Frontal Gyrus	6.91	36	50	28	
	5.76	-38	42	16	
Middle Frontal Gyrus,					L
Inferior Frontal Gyrus	4.77	-42	20	28	
Inferior Temporal Gyrus/					L
Temporal Occipital					
Fusiform Cortex	6.42	-48	-58	-22	
Inferior Temporal Gyrus/					R
Temporal Occipital					
Fusiform Cortex	5.66	46	-38	-14	

Lateral Occipital Cortex/ Occipital Fusiform Gyrus	6.21	-32	-86	-18	L
Lateral Occipital Cortex/ Precuneous Cortex	5.09	12	-68	50	R
Lateral Occipital Cortex/ Precuneous Cortex	4.69	12	-68	54	R
Thalamus	6.04	-12	-24	4	R/L
	4.94	-18	-24	0	
	4.87	8	-24	6	
	4.79	16	-10	6	
	4.73	16	-12	-2	
Occipital Pole	5.37	22	-98	-4	R
Putamen	5.03	-32	-2	-4	R/L
	5.02	22	2	14	
Brain Stem	4.95	-4	-36	-22	R/L
Frontal Operculum Cortex	4.84	30	26	12	R
SMA, Superior Frontal Gyrus	4.75	-10	-2	64	L

4.3.3. *Neurofeedback Training*

After rt-fMRI-nf training (contrast of run 4 > 1), relative to the CG, the EG showed increased activation in the left DLPFC ROI in the Frontal Pole/Middle Frontal Gyrus (peak x/y/z = -28/40/34; Z = 5.43; *Figure 9*) and in the bilateral ACC ROI in the ACC/ Paracingulate Gyrus (peak x/y/z = -6/8/38; Z = 18.3; Figure 9A). In the left DLPFC ROI, there was a region in the Superior/Middle Frontal Gyrus (peak x/y/z = -20/32/38; Z = 8.01; Figure 9) that showed reduced activation in the EG relative to the CG (Table 7). The CG did not show significant activation changes in these areas over rt-fMRI-nf runs (run 4 > run 1).

Table 7

Regions and MNI coordinates for areas with increase in activation from run 1 to run 4 in the EG compared to the CG in the left DLPFC and ACC ROI.

			MNI coordinates (mm)			
	Area	**Z value**	x	y	z	
EG > CG	ACC	18.3	-6	8	38	L
	ACC	9.86	-6	6	48	L
	ACC	8.16	-12	14	38	L
	ACC	7.88	10	30	42	R
	ACC	7.65	-10	26	36	L
	ACC	7.49	-8	16	44	L
	ACC	7.11	-10	28	24	L
	ACC	6.8	-6	16	38	L
	ACC	6.78	4	40	36	R
	ACC	5.71	0	16	46	
	ACC	5.71	-10	26	40	L
	DLPFC	5.43	-28	40	34	L
	ACC	4.91	-10	18	48	L
	DLPFC	4.63	-22	46	26	L
	DLPFC	4.55	-32	40	38	L
	ACC	4.54	4	2	44	R
	DLPFC	4.23	-26	46	28	L
	ACC	4.17	-12	30	28	L
	ACC	3.74	-4	20	28	L
	DLPFC	3.67	-28	42	40	L
	ACC	3.66	-4	14	52	L
	ACC	3.42	-4	32	48	L
	DLPFC	3.31	-36	50	14	L
	ACC	3.29	-6	44	34	L
CG > EG	DLPFC	8.01	-20	32	38	L

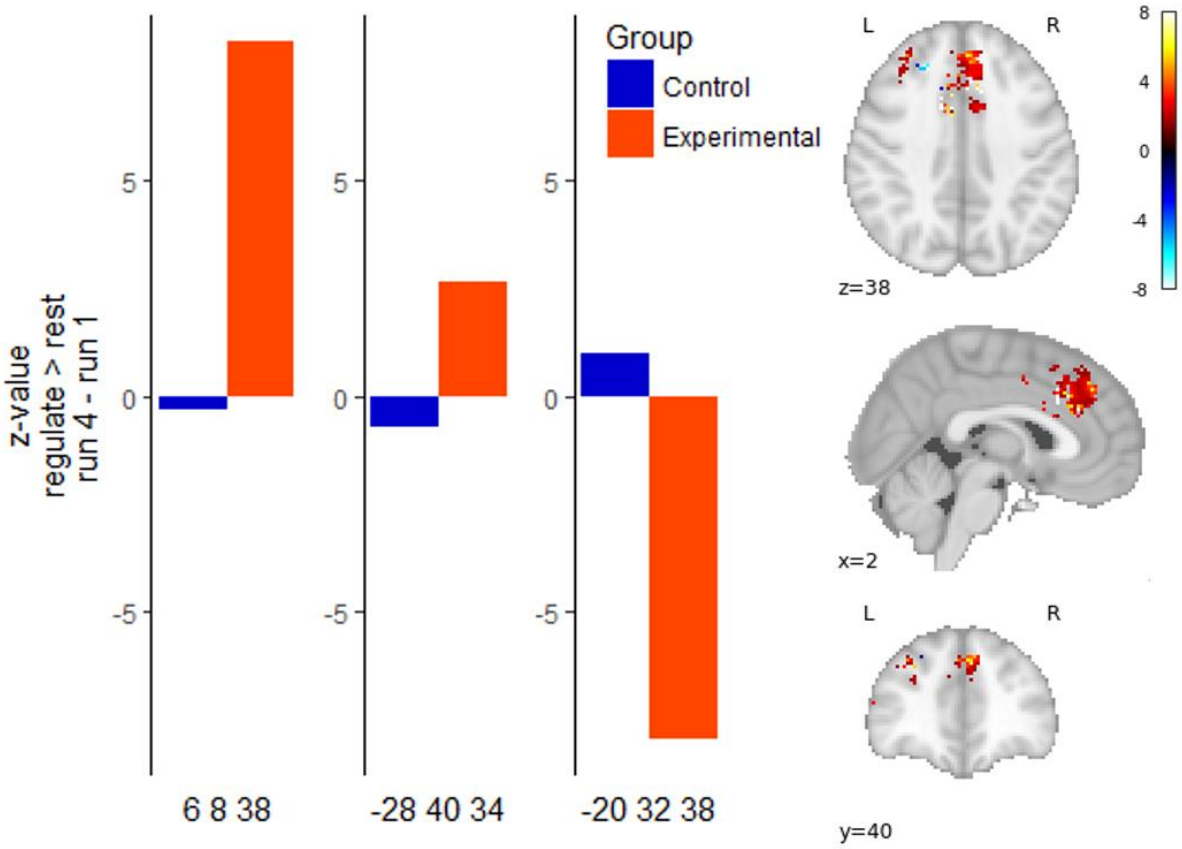

Figure 9. Increased (red) and decreased (blue) activation in the EG relative to the CG (rt-fMRI-nf run 4 > run 1 in the contrast regulate > rest) in the bilateral ACC and left DLPFC ROIs. Results are Z-maps displayed at a threshold of $p < .05$ uncorrected for illustrative purposes.

Furthermore, reductions in DASS Anxiety scores in the EG were positively associated with activation in the left DLPFC ROI in the Middle Frontal Gyrus (peak x/y/z/ = -52/22/32; Z = 4.63) and Inferior Frontal Gyrus (peak x/y/z = -54/26/10; Z = 3.72) and in the bilateral ACC ROI in the left Paracingulate Gyrus (peak x/y/z = -4/22/38; Z = 4.25), left Medial Superior Frontal Gyrus (peak x/y/z = 4/22/54; Z = 4.08) and left ACC (peak x/y/z = -8/32/24; Z = 3.81). Reductions in DASS Anxiety scores were negatively associated with activation in the left DLPFC ROI in the Frontal Pole (peak x/y/z = -24/56/16; Z = 4.94) and in the bilateral ACC ROI in the Supplementary Motor Area (SMA, peak x/y/z = -10/4/42; Z = 4.98; Figure 10; Table 8)

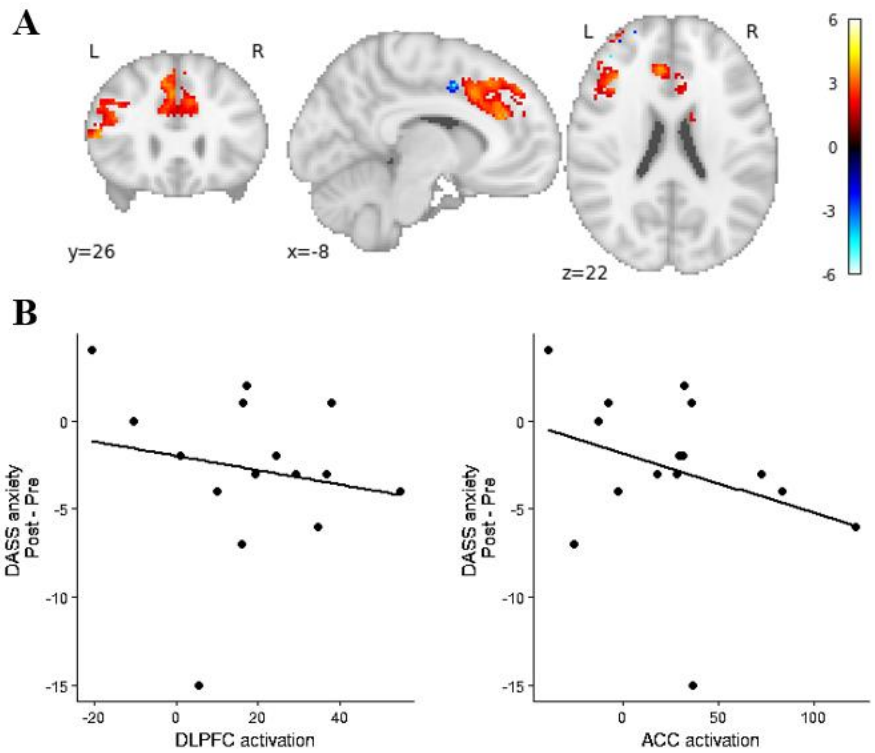

Figure 10. Regression between left DLPFC and bilateral ACC ROI activation and changes in DASS anxiety scores over rt-fMRI-nf training in the EG. (A) Positively (red) and negatively associated areas (blue). *Results are Z-maps displayed at a threshold of $p < .05$ uncorrected for illustrative purposes.* (B) Scatter plot between changes in DASS anxiety and extracted parameters from peak voxels (based on 6 mm spheres).

Table 8

Regions and MNI coordinates in the ROI that are associated with DASS anxiety decreases during rt-fMRI-nf training. ($p_{FWE\ peak} < .05$, local maxima).

			MNI coordinates (mm)		
	ROI	**Z-Value**	x	y	z
Decreased brain activation	ACC	4.98	-10	4	42
	DLPFC	4.94	-24	56	16
	DLPFC	4.65	-40	42	22
Increased brain activation	DLPFC	4.63	-52	22	32
	ACC	4.25	-4	22	38
	ACC	4.08	4	22	54
	DLPFC	3.94	-38	22	24
	ACC	3.81	-8	32	24
	ACC	3.81	6	44	32
	ACC	3.72	-12	14	36
	DLPFC	3.72	-54	26	10
	DLPFC	3.72	-36	28	22
	DLPFC	3.66	-50	26	10

4.3.4. *Functional Connectivity during Neurofeedback Training: PPI*

Relative to the CG, functional connectivity between the left DLPFC ROI seed region and the bilateral ACC ROI was increased over rt-fMRI-nf training runs (run 4 > run 1) in the EG (peak x/y/z = -6/34/26; Z = 5.16). There was also decreased functional connectivity (run 4 > 1) in the EG compared to the CG in between the left DLPFC seed region and the SMA (x/y/z = -12/0/44; Z = 4.59). (*Figure 11*, Table 9)

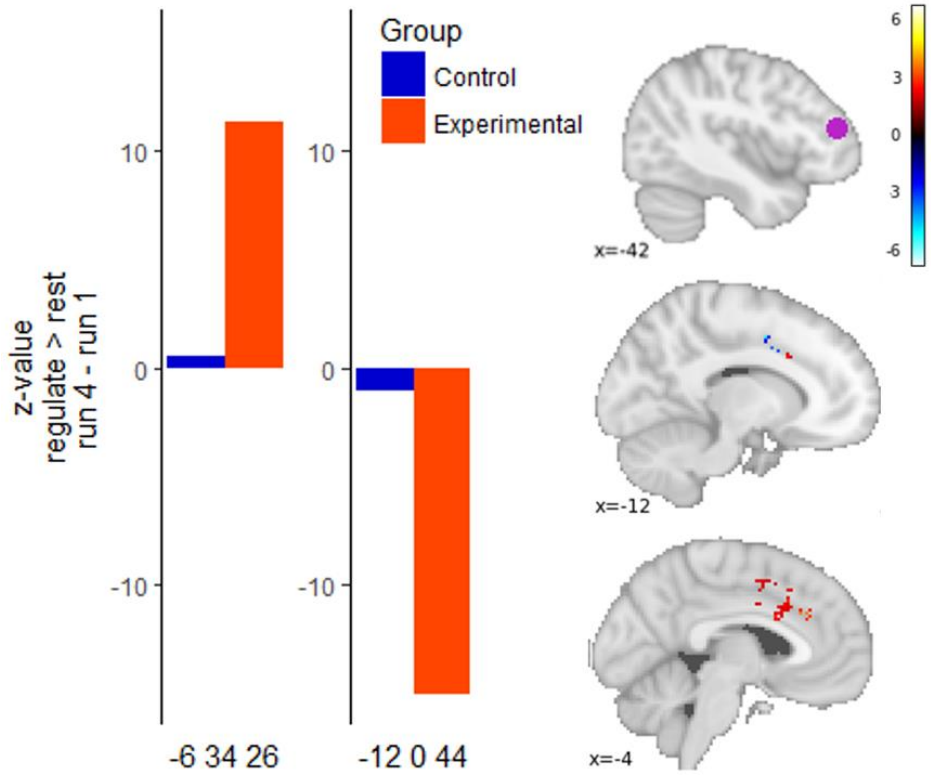

Figure 11. PPI analysis using left DLPFC seed region (purple) showing increased (red) and decreased (blue) functional connectivity in bilateral ACC ROI. Results are Z-maps displayed at a threshold of $p < .05$ uncorrected for illustrative purposes.

Table 9

MNI coordinates and Z values with increased task specific functional connectivity from run 1 to run 4 in the EG compared to the CG with the bilateral ACC ROI.

		MNI coordinates (mm)		
	Z value	x	y	z
EG > CG	5.16	-6	34	26
	4.71	6	30	22
	3.80	0	32	38
CG > EG	4.59	-12	0	44
	3.97	-8	0	40

Regression analysis showed that in the EG and within the ACC ROI, changes in DASS anxiety scores were positively associated with increased functional connectivity in the bilateral ACC/paracingulate sulcus (peak left x/y/z = -10/28/36; Z = 4.31, peak right x/y/z = 8/40/36; Z = 4.15) and with reduced functional connectivity in a more inferior region of the bilateral ACC ROI (peak x/y/z = -4/32/28; Z = 4.25; *Figure 12*;

Table 10)

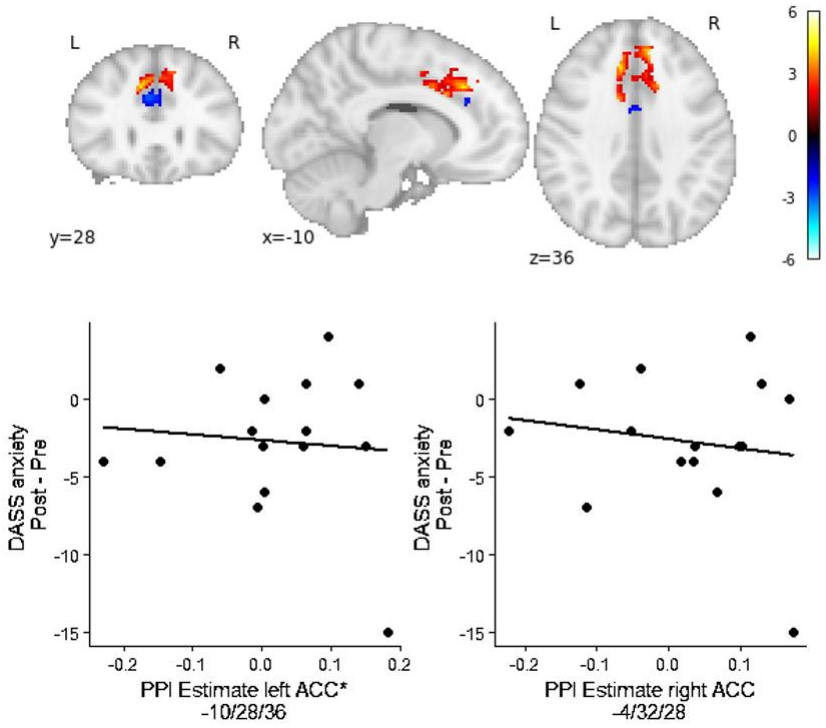

Figure 12. Regression between PPI estimate of changes in functional connectivity between left DLPFC seed region and bilateral ACC ROI and changes in DASS Anxiety scores over rt-fMRI-nf training in the EG. Brain map shows positively (red) and negatively associated areas (blue). *Results are Z-maps displayed at a threshold of p < .05 uncorrected for illustrative purposes.* Scatter plot showing association between changes in DASS anxiety scores (Post – Pre training) and extracted PPI parameters from peak voxels in the ACC (based on 6 mm sphere).

Table 10

Regions and MNI coordinates in the bilateral ACC ROI where PPI parameters are associated with DASS anxiety decreases during rt-fMRI-nf training. ($p_{FWE\ peak}$ < .05, local maxima).

		MNI coordinates (mm)		
	Z-Value	x	y	z
Decreased connectivity	4.25	-4	32	28

Increased	4.31	-10	28	36
connectivity	4.15	8	40	36
	3.83	6	24	40
	3.84	8	36	36
	3.71	8	22	50

4.4. Discussion

Using a controlled experimental repeated-measures design, the potential of connectivity-based rt-fMRI-nf for enhancing activity and connectivity in attentional control networks and reducing anxiety levels in HTA individuals was examined. Functional connectivity-based rt-fMRI-nf was implemented with a customised sliding-window approach [123], which allowed participants to monitor and regulate dynamic functional connectivity in real-time. Functional connectivity between left DLPFC and bilateral ACC was targeted with the rt-fMRI-nf, as coupling between these regions is known to be important for attentional control and has been shown to be reduced in people with high levels of trait anxiety [27, 82]. A functional localiser task, which provoked activation in left DLPFC and ACC, was used to define rt-fMRI-nf target regions.

First, PPI analysis showed that HTA individuals could successfully enhance functional connectivity between the left DLPFC and bilateral ACC when provided with veridical rt-fMRI-nf compared to sham feedback. Importantly, in the EG, increased functional connectivity between the DLPFC and ACC was associated with reduced anxiety levels over the rt-fMRI-nfb training period. However, in a more inferior region of the ACC ROI, an association between reduced DLPFC - ACC

functional connectivity and decreased anxiety levels was observed. Second, relative to the CG, the EG showed a decrease in anxiety levels post-rt-fMRI-nf training that was not seen in the CG. This effect appeared to be specific to anxiety levels as no post-training effects were seen for depression and stress levels. Third, rt-fMRI-nf training led to both increased and decreased functional activation in different sub regions of the left DLPFC ROI, and increased activity in the bilateral ACC ROI in the EG relative to the CG. Furthermore, increased functional activation in ACC and left DLPFC during rt-fMRI-nf training was associated with decreased anxiety levels. However, areas in the bilateral SMA and left Frontal Pole showed decreased connectivity, and activity that was associated with decreased anxiety levels.

Together, these results show that participants in the EG were able to self-regulate functional connectivity, guided by veridical rt-fMRI-nf, resulting in altered functional activity and connectivity in attentional networks that were associated with reduced anxiety levels.

The theoretical framework of ACT predicts inefficient task processing in people with HTA. Based on previous literature, it appears that this inefficiency may be underpinned by a lack of aberrant activation and connectivity of DLPFC and ACC in people with high levels of trait anxiety and worry (e.g., [13, 14, 34, 71]). This study provides preliminary evidence that people with HTA have the ability to alter their brain activity with the aid of rt-fMRI-nf training. Furthermore, up-regulating or increasing DLPFC-ACC connectivity was associated with a reduction in

self-reported anxiety, pointing towards a mechanistic and causal link between efficient functioning in attentional control networks and anxiety levels. ACT focuses on the effects of trait anxiety on attentional control, while there is increasing understanding, that there may be a bi-directional relationship between attentional control and trait anxiety (i.e. changes in attentional control processes can also influence anxiety levels). Importantly however, these findings must be considered in conjunction with potential changes in performance in attentional control tasks, assessing whether the observed changes in brain activation and connectivity (i.e. *processing efficiency*) are accompanied by respective improvements in performance (i.e. *performance effectiveness*; Chapter 5).

Rt-fMRI-nf training of functional connectivity is a very recent development; nevertheless, recent findings in this field, as well as this study, demonstrate that participants can successfully learn to regulate functional connectivity between two brain areas (e.g., [104, 122]). Given these initial findings, using rt-fMRI-nf training of functional connectivity is a promising direction for rt-fMRI-nf to be explored further in the future, so are other approaches of modulating connectivity using rt-fMRI-nf (e.g., effective connectivity-based rt-fMRI-nf [105, 128]).

Furthermore, rt-fMRI-nf has been shown to be a viable method to reduce anxiety, using a variety of target regions and mechanisms. While most rt-fMRI-nf studies aiming to improve anxiety use the amygdalae or networks including the amygdalae as rt-fMRI-nf targets (e.g., [114, 122]), only few studies target underlying neurocognitive processes. Zilverstand and

colleagues [167] present the first such study; they aimed to improve anxiety regulation in patients with spider phobia to subsequently reduce anxiety. To this end, they provided participants with feedback on insula activity, a region heavily implied in cognitive regulation of anxiety, and report reduced anxiety as a function of successful regulation. Similarly, the results presented here show reductions in anxiety as a result of rt-fMRI-nf training of functional connectivity that is important for attentional control, a cognitive process that has been linked to HTA. If anxiety is largely defined by cognitive characteristics, such as worry [3], then interventions need to be aiming at the processes underlying these.

In addition to rt-fMRI-nf-related increases in functional connectivity and activity, there was reduced functional connectivity between the left DLPFC and a SMA region that fell within the bilateral ACC ROI. Whilst the SMA is anatomically close to the ACC, it is a distinct area within a distinct RSFC network that is usually reported as being negatively associated with DLPFC activity [230], although more anterior parts of the Dorsomedial Cortex may be positively associated with DLPFC activity [231, 232]. Therefore, it is possible that increased DLPFC-ACC connectivity due to rt-fMRI-nf training, also resulted in a reduced functional connectivity between the DLPFC and SMA. Furthermore, reduced functional connectivity between the DLPFC seed region and a small area of the ACC was also associated with a reduction in anxiety levels. Whilst the reasons for this result are unclear it is likely that the ACC ROI contained functionally distinct areas of the medial cortex that may have responded differently to rt-fMRI-nf training. Moreover, a region

within the left DLPFC ROI (in the Superior/Middle Frontal Gyrus) showed reduced activity over the rt-fMRI-nf training period. Again, the reasons for reduced activity in this DLPFC region are not clear but it is possible that as left DLPFC-ACC functional connectivity increases, parts of the DLPFC may act more efficiently with this network [13] resulting in reduced activity over the rt-fMRI-nf training period.

4.4.1. *Limitations*

While the sample size here is comparable to other rt-fMRI-nf studies in healthy populations (see [109, 111]), this study was only powered to detect medium to large effect sizes. Thus, however promising these results, they need to be interpreted with some caution and replication in a larger sample is needed.

Furthermore, it is important to acknowledge the possibility that some of the effects observed in this experiment may be due to the participants attempt to self-regulate brain activation rather than true self-regulation of functional connectivity between the ACC and DLPFC. Emmert and colleagues [109] report a distinct pattern of brain activity that is associated with attempts of self-regulation that is independent of target region and direction of regulation. Nevertheless, the randomised controlled nature of the study and the specificity of the effects to the EG suggest that these results are likely a consequence of successful self-regulation of the targeted functional connectivity.

To date, functional connectivity-based rt-fMRI-nf has been applied in very few studies [104, 122], and the exact protocols used differ considerably.

The approach proposed here is highly standardised, yet customised to each participant and it can easily be replicated and adapted for future research.

4.4.2. *Conclusions*

In conclusion, this study demonstrates the feasibility of using connectivity-based rt-fMRI-nf training (based on functional connectivity between left DLPFC and the ACC) to reduce anxiety levels and alter activity and connectivity in the left DLPFC and bilateral ACC. These neural findings could be interpreted as a pattern of increased efficiency in brain circuitry important for attentional control, which led to reduced levels of anxiety. These results need to be replicated in larger samples, and more work is needed to better understand the relationship between efficient processing in attentional control networks and anxiety levels. Specifically, such research should focus on the bi-directional influences of anxiety on attentional control and vice versa.

5. Influence of rt-fMRI-nf Training on Attentional Control

5.1. Introduction

Attentional bias occurs when individuals show increased vigilance towards threatening stimuli and therefore allocate disproportionate amounts of attention to these stimuli [233]. Increased attentional bias to threat is commonly observed in people with HTA [8]. However, cognitive impairments in HTA are not limited to threat-related stimuli [7]. A main characteristic of trait anxiety is excessive worry [3], described by PET [19] and ACT [7] as the main cognitive component of trait anxiety. ACT outlines how worry leads to impaired attentional control by competing for limited cognitive resources, and by increasing the salience of threat-related stimuli, thus leading to attentional bias [7].

Several psychological interventions, designed to train or modify attention, have been developed specifically for people with high anxiety [222-224]. Frequently, the focus of such training is to modify attentional bias towards threat-related stimuli. The two most common types of intervention are targeting attentional selectivity, in which participants are taught to avoid threat-related stimuli, or more complex interventions targeting interpretive bias [234]. Despite mixed results of interventions targeting attentional bias [222, 223], there is evidence that training of attentional control can be an effective treatment for anxiety disorders (e.g., [224, 234]); whereby an improvement in attentional control predicts reduced anxiety [224]. The mixed evidence for these interventions can partly be attributed to flaws in experimental design (e.g., choice of measure, choice of control condition;

[223, 224]), but may also be a reflection that merely modifying attentional bias towards threat-related stimuli, rather than training more global attentional processes, is insufficient.

Brain imaging research has shown that two regions, which are most prominently associated with impairments in attentional control in people with HTA, are the DLPFC and ACC (e.g., [6, 13, 62, 70, 72]). Specifically, functional connectivity between these two regions has been shown to be decreased in people with HTA, which has been linked to impaired task performance (e.g., [13, 34]). Having identified this key mechanism, underlying effective and efficient task processing, modulating functional connectivity between the DLPFC and ACC in people with HTA using rt-fMRI-nf could lead to improved effectiveness and efficiency in attentional control.

Preliminary studies have attempted to use rt-fMRI-nf for cognitive enhancement and as an intervention to ameliorate cognitive impairments [169]. Findings show that in principle, using rt-fMRI-nf is a feasible approach to improve attentional control task performance. However, it is important to consider that this approach may not be effective in all participants, and that generally effect sizes have been small. Nevertheless, some of these limitations may be due to the relatively small sample sizes and short training protocols employed in early studies [169].

Rt-fMRI-nf has been used specifically to improve higher cognitive functions, such as working memory and attentional control. Two sessions of rt-fMRI-nf on activity in the left DLPFC significantly improved

performance in a digit span test [235]. In addition deBettencourt and colleagues [102] have demonstrated in a controlled experiment, that participants show improvements in sustained attention after just one session of rt-fMRI-nf, targeting relevant brain patterns (individually defined in each participant using a data-driven approach).

In the current study, it was sought to examine if rt-fMRI-nf training can improve attentional control performance, measured using a range of offline cognitive tasks. Three tasks were used to measure different aspects of attentional control performance; firstly a Continuous Performance Task (CPT) to measure sustained attention ability, secondly an EPT measuring attentional bias to emotionally salient stimuli, and finally a colour-word Stroop task, predominantly requiring the inhibition function of attentional control. ACT predicts impaired performance of all the aforementioned attentional control functions [7], which has been confirmed in empirical studies [17].

It is hypothesised that veridical DLPFC-ACC functional connectivity-based rt-fMRI-nf training will lead to improved attentional control performance post-rt-fMRI-nf training. This improvement is hypothesised to be apparent both with and without threat-related stimuli.

5.2. **Methods**

The study sample, experimental design and rt-fMRI-nf protocol used in this experiment are described in detail in Chapter 3.

5.2.1. *Cognitive Tasks*

To assess the effects of rt-fMRI-nf training on attentional control, three tasks were used to assess sustained attention, inhibition and attentional bias, at pre- and post-rt-fMRI-nf time points. All tasks were customised and programmed using Python and presented in PsychoPy (Pierce, 2007).

5.2.1.1.Continuous Performance Task

Sustained attention and response inhibition, both aspects of attentional control [7], were measured with the AX-CPT [236, 237]. During this task, participants must maintain their attentional focus over a period of 12 minutes to respond to visually presented target letters (always X, "X") that follow a cue letter (always A, "A"), while inhibiting response to non-target letters (any letter, but not A or X, "O"). Target and non-target trials appear intermixed in a pseudorandom sequence (70% target, 30% non-target) and there were a total of 150 trials. Four types of trials were differentiated; AX target trials (70%) and AO (10%), OX (10%) and OO (10%) non-target trials. Both RTs and ERs were measured as both have been found to be useful indicators of attentional control ability [236]. ERs were based on the proportion of false positive responses to non-target trials and false-negative responses to target trials. RTs were only assessed for target trials, so for this measure no comparison between Conditions, but merely between groups (EG, CG) and time point (pre, post) was performed.

Participants were instructed to respond as quickly and accurately as possible to the target letter (i.e. X), if it immediately followed the cue letter (i.e. A). In the task, letters were presented sequentially for 500 ms followed a fixation period of a random duration between 1600 ms and 2400 ms, on average 2000 ms. Responses to the letters were recorded for 1800 ms from stimulus onset; participants responded by pressing spacebar (see Figure 13).

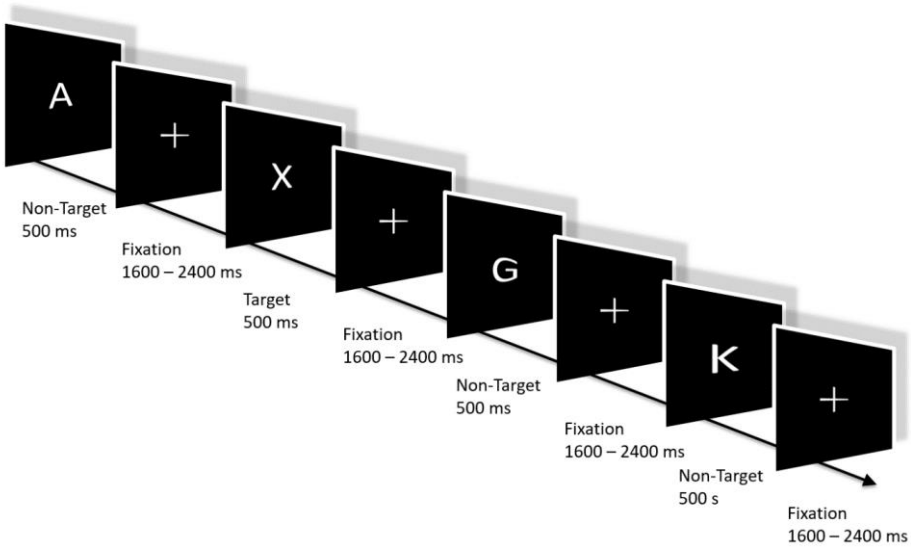

Figure 13. Continuous Performance Task Paradigm (not to scale).

A correct response was recorded when participants pressed spacebar to the target letter X, if this was preceded by the cue letter A; or in all other circumstances when no response was given. Incorrect responses were recorded when participants failed to respond to target AX trials, or responded to non-target trials.

5.2.1.2.Emotional Probe Task

The degree of attentional bias to emotionally salient stimuli (i.e. positive, socially threatening, and physically threatening) was established using an

EPT. Dot-probe tasks are widely used to assess attentional bias and evaluate the effectiveness of attentional bias modification using either word or picture stimuli (e.g., [224, 234]). A Meta-Analysis showed no significant difference between word and picture stimuli in producing attentional bias to threat in anxious populations [238]. Using a dot-probe paradigm, the EPT measures attentional bias for socially threatening (ST), physically threatening (PT), positive (PO) and neutral (NT) words. There were 24 trials per condition in random order over a period of 4 minutes and 16 seconds in which participants were required to inhibit emotional distractor information before responding to a probe. There were congruent and incongruent trials dependent on whether probe and emotional word were on the same or opposite side of the screen, these were counterbalanced. RTs and ERs were measured to assess participants' ability to inhibit irrelevant emotional information.

Participants were instructed to respond as quickly and accurately as possible to a probe. At the beginning of each trial, two distractor words appeared on either side of a fixation cross; emotional words were matched with neutral words of similar length. As a control condition two neutral words were paired. Emotional words were counterbalanced between the left and right side of the screen. The words disappeared after 1000 ms, and a probe appeared in the position of one of the words for 1100 ms, which was also the maximum time to respond to the probe. Participants had to respond with either the left or right arrow key depending on the side of the probe. The inter trial interval (ITI) after every trial was a random interval

from 250 ms to 750 ms, with a mean of 500 ms and a standard deviation of 150 ms (

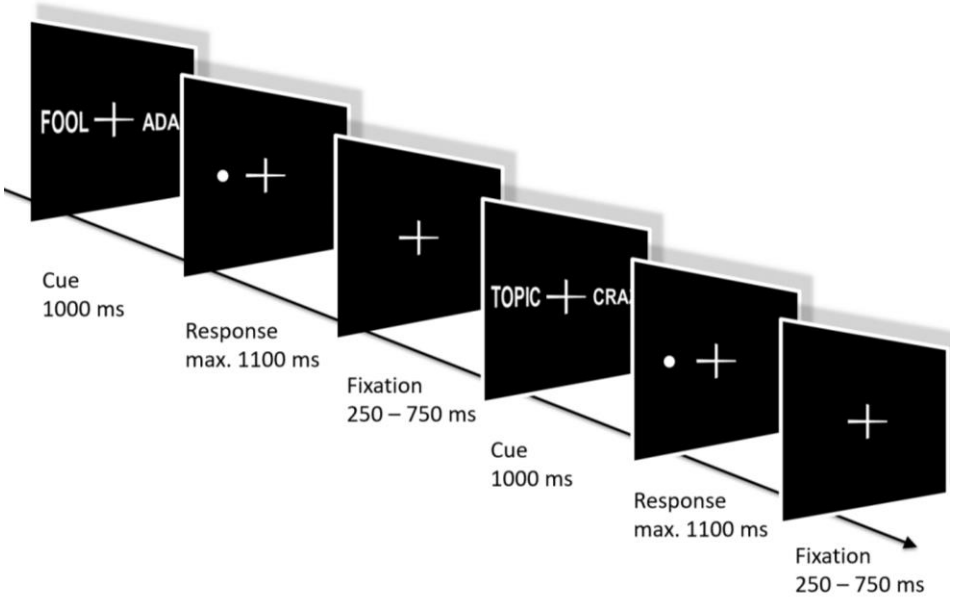

Figure *14*).

Figure 14. Emotional Probe Task Paradigm (not to scale).

A correct response was recorded when participants pressed the arrow key, corresponding to the side of the probe, incorrect responses were recorded when the wrong key was pressed, or no response was given. RT was recorded for all trials.

5.2.1.3.Stroop Task

Participants performed a colour-word Stroop task [184], to measure the inhibition function of attentional control. Participants responded with one of four fingers of their right hand to the font colour (Red, Blue, Green, & Yellow) of the colour word presented in the middle of the screen (Red, Blue, Green, & Yellow). The presentation time for each stimulus was 1000 ms and participants were allowed 2000 ms from stimulus onset to give a

response (i.e. responses were registered from the onset of each stimulus trials). Participants were instructed to respond as quickly and as accurately as possible while RTs and ERs were recorded. The task consisted of 48 Congruent (colour word and font colour did match) and 48 Incongruent (colour word and font colour did not match) trials. Trials were presented in a randomised order and each trial took between 4000 and 6000 ms (random ITI from 2000 to 4000 ms with a mean of 3000 ms, and a SD of 500 ms).

5.2.2. *Data Analysis*

Task data were analysed using ANOVA in R 3.4.3 (R Core Team, 2017) and a significance threshold of $p < .05$ was applied.

5.2.2.1.Continuous Performance Task Performance

Three participants were not included in the analysis of the CPT, as task data was not available for both time points due to participants not following the instructions to the task, hence the sample size in this task was n = 27 (EG =15, CG =12). Each participant's mean ER and RT for the CPT was calculated for each condition (AX, AO, OX, OO), and for each time point (T1, T2). Mixed ANOVA were performed for both RT and ER data. RTs for target trials were analysed with the between-subjects factor group (EG, CG) and time point (T1, T2) as a within-subjects factor. The mixed ANOVA for ERs included the between-subjects factor group and time point and condition as within-subjects factors. Significant results were explored further with pairwise comparisons.

5.2.2.2.Emotional Probe Task Performance

Similar to the analysis of the CPT data, mean ERs and RTs from the EPT data were calculated for each condition and time point, they were simplified further by subtracting the mean ER and RT for incongruent from congruent trials, resulting in scores for attentional bias for ERs and RTs. The ER and RT data were analysed in two mixed ANOVAs with the between-subjects factor group (EG, CG) and time point (T1, T2) and condition (ST, PT, NT, PO) as within-subjects factors. Significant results were explored further with pairwise comparisons.

5.2.2.3.Stroop Task Performance

Participants' mean ERs and RTs for the Stroop task were calculated for each condition and time point. Two mixed ANOVAs, one for ER and on for RT were performed. Within-subjects factors were task condition (Congruent, Incongruent) and time point (T1, T2); furthermore, group (CG, EG) was included as a between-subjects factor. Significant results were explored further with pairwise comparisons.

5.3.**Results**

5.3.1. *Continuous Performance Task*

Two measures of performance were assessed in the Continuous Performance Task. Firstly RTs for target trials were compared across groups and time points. ANOVA revealed a non-significant effect of group ($F(1, 25) = 0.32$, $p = .579$), although there was a significant effect for time point ($F(1, 25) = 8.90$, $p = .006$, $\eta_{part}^2 = .263$) with RTs being faster post- compared to pre-rt-fMRI-nf training. The interaction between time point

and group was non-significant (F(1, 25) = 0.11, p = .740). Figure 15 shows the mean RTs between groups and time points.

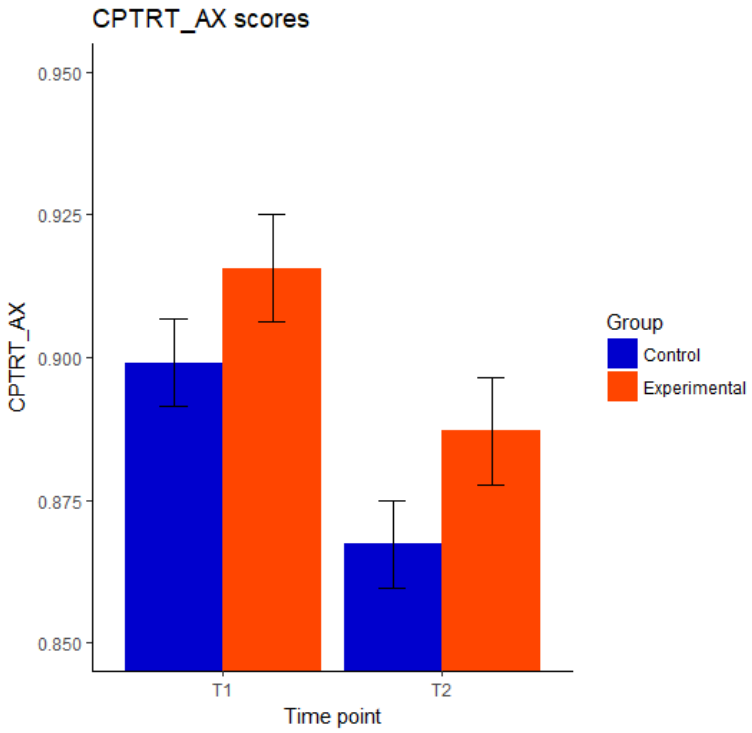

Figure 15. Reaction Times in target trials during the Continuous Performance Task by Group and Time Point.

Secondly, ERs were compared across groups, conditions and time points. ANOVA revealed a significant effect for task condition (F(3, 75) = 18.06, p < .001, ηpart² = .419). However, the effects of group (F(1, 25) = 0.02, p = .891) and time point (F(1, 25) = 0.30, p = .591) were both non-significant, as were the interaction effects between group and task condition (F(3, 75) = 0.13, p = .940), between group and time point (F(1, 25) = 0.40, p = .533), between task condition and time point (F(3, 75) =

0.29, p = .618), and the three-way interaction between group, task condition and time point (F(3, 75) = 0.89, p = .452; *Figure 16*).

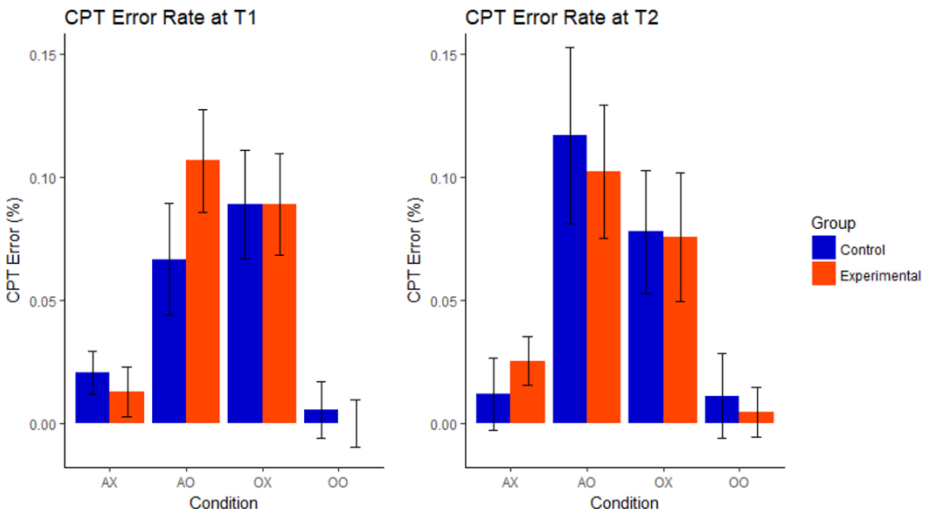

Figure 16. Error Rate in the Continuous Performance Task by Condition. (A) Session 1. (B) Session 2.

5.3.2. *Emotional Probe Task*

Differences in RTs between incongruent and congruent trials were compared across groups, conditions and time points. ANOVA revealed a significant effect for task condition (F(3, 84) = 2.91, p = .040, η_{part}^2 = .094). However, the effects of group (F(1, 28) = 0.01, p = .904) and time point (F(1, 28) = 0.07, p = .795) were both non-significant, as were the interaction effects between group and task condition (F(3, 84) = 0.38, p = .769), and between group and time point (F(1, 28) = 0.19, p = .663). Although the interaction between task condition and time point was significant (F(3, 84) = 3.29, p = .025, η_{part}^2 = .105), showing increased bias in the ST and PT conditions and reduced bias in the NT and PO conditions over time. The three-way interaction between group, task condition and time point was non-significant (F(3, 84) = 0.51, p = .675). Differences in

ERs between incongruent and congruent trials were compared across groups, conditions and time points. ANOVA revealed no significant effect for task condition ($F(3, 84) = 1.20$, $p = .315$), group ($F(1, 28) = 0.60$, $p = .610$) and time point ($F(1, 28) = 0.43$, $p = .519$). Neither the interaction between group and task condition ($F(3, 84) = 1.51$, $p = .219$), nor the interaction of task condition and time point ($F(3, 84) = 1.83$, $p = .148$) were significant. However, there was a significant interaction between group and time point ($F(1, 28) = 5.24$, $p = .030$, $\eta_{part}^2 = .158$). The EG had reduced bias scores towards emotionally salient stimuli over time, while the CG had higher bias scores over time. The three-way interaction between group, task condition and time point was not significant ($F(3, 84) = 0.28$, $p = .840$; *Figure 17*).

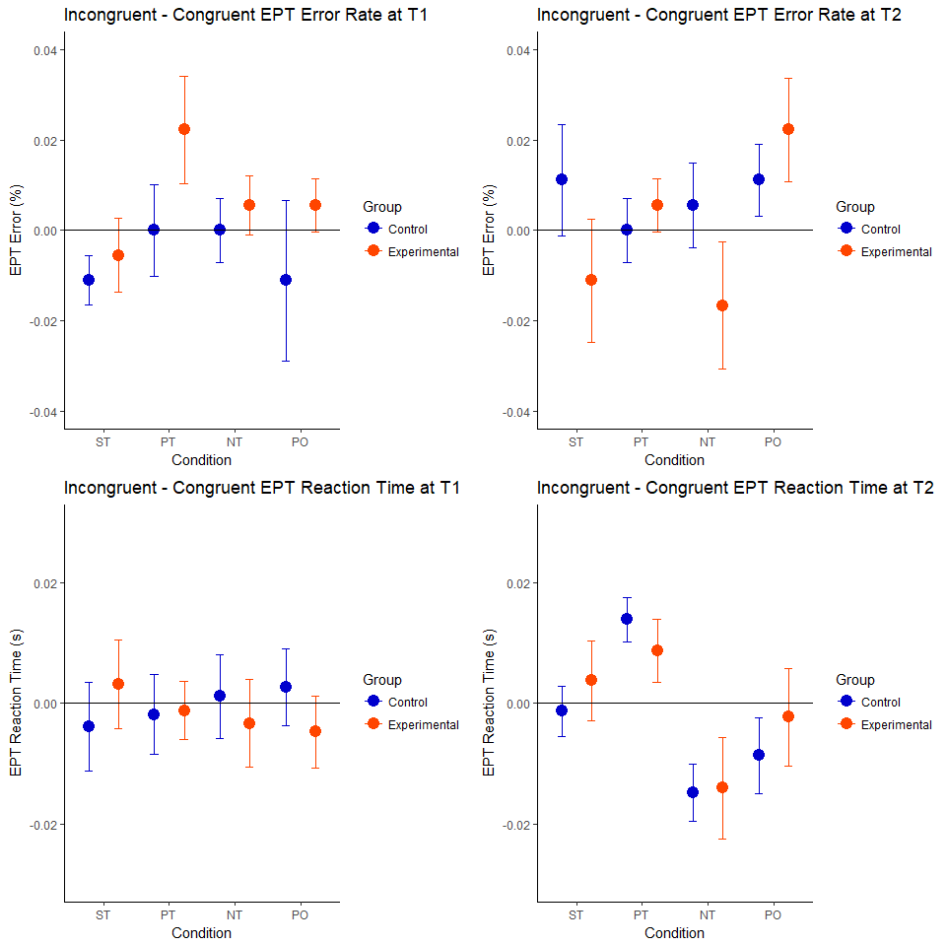

Figure 17. Error rates and reaction times in the EPT.

5.3.3. *Stroop Task Performance*

For the Stroop Task data, ANOVA revealed a significant effect for task condition ($F(1, 28) = 15.60$, $p < .001$, $\eta part^2 = .358$) with greater RT during incongruent trials and a significant effect of time point ($F(1, 28) = 108.69$, $p < .001$, $\eta part^2 = .795$), revealing an improvement in RT at T2 across groups and task conditions. However, the three-way interaction between group, task condition and time point ($F(1, 28) = 0.41$, $p = .526$) was non-significant. This shows that RTs for incongruent trials did not significantly improve in the EG relative to the CG post-rt-fMRI-nf training (Table 11). For ER, ANOVA also revealed a significant effect of task condition (F(1,

28) = 6.64, p = .016, ηpart² = .192) with consistently greater ER in the incongruent condition. However, the effects of group (F(1,28) = 0.35, p = .562) and time point (F(1,28) = 0.93, p = 344) were both non-significant, as was the three-way interaction between group, task condition and time point (F(1,28) = 0.48, p = .493). This shows that ER for incongruent trials did not significantly improve in the EG relative to the CG post rt-fMRI-nf (see Table 11).

Table 11

Means and SDs in the Stroop Task, by outcome measure, Time Point, Condition and Group.

			Group	
Measure	**Time Point**	**Condition**	EG	CG
RT	PRE	Congruent	0.80 (0.17)	0.87 (0.19)
		Incongruent	0.93 (0.19)	0.97 (0.20)
	POST	Congruent	0.75 (0.13)	0.77 (0.15)
		Incongruent	0.86 (0.19)	0.88 (0.22)
ER	PRE	Congruent	0.06 (0.06)	0.08 (0.07)
		Incongruent	0.07 (0.07)	0.09 (0.10)
	POST	Congruent	0.05 (0.09)	0.05 (0.04)
		Incongruent	0.09 (0.11)	0.07 (0.09)

5.4. **Discussion**

The aim of this experiment was to investigate the feasibility of using DLPFC-ACC functional connectivity-based rt-fMRI-nf in people with HTA to improve attentional control. This study tested if the neural effects of rt-fMRI-nf training targeting attentional control regions transferred to an improvement in *performance effectiveness*. Specifically, tasks that measure inhibition, sustained attention and attentional bias were used. This was because these aspects of attentional control have consistently been

shown to be impaired in people with high levels of trait anxiety [17] resulting in attentional bias to negative stimuli that may maintain anxiety levels and also impaired attentional control in the absence of threat-related stimuli [7].

Using a measure of sustained attention (CPT), RTs for both EG and CG improved at the post- relative to pre-rt-fMRI-nf time point. As there was no significant group x time point interaction it is likely that improved (i.e. faster) RTs seen at the post-rt-fMRI-nf time point were due to practice effects and not specific to effects of rt-fMRI-nf targeting DLPFC-ACC functional connectivity. There were also no relevant effects on ERs in this task. These findings did not support the prediction of improved *performance effectiveness* in cognitive control tasks after rt-fMRI-nf.

Consistent with the hypotheses, during the EPT the EG did show reduced attentional bias at the post- relative to pre-rt-fMRI-nf training time point, compared to the CG. ERs on the dot-probe task, a measure of attentional bias towards emotionally salient stimuli, were reduced in the EG post training but not in the CG. This suggests that functional connectivity-based rt-fMRI-nf on DLPFC-ACC connectivity tracked with reduced attentional bias to threat-related stimuli. In the same task, participants showed greater bias towards threat-related stimuli in RTs over time, suggesting increased attentional bias. However, this effect was the same for the EG and the CG so cannot be attributed to the effects of rt-fMRI-nf training. It should be noted that dot-probe paradigms are the most common measure to evaluate changes in attentional bias in highly anxious populations [224]. However,

effect sizes are typically much larger than reported here, therefore this result must be interpreted with much caution.

Finally, in a task assessing response inhibition (Stroop Task), both groups improved their RTs but not ERs at the post- relative to pre-rt-fMRI-nf time point. This is likely to be due to practice effects. There were no group specific changes in RT over time; however, there was a trend toward improved ERs in the CG relative to the EG, which is inconsistent with the hypothesised improvement of attentional control in the EG.

Using rt-fMRI-nf for cognitive enhancement is a very recent development [169]. It is not uncommon, that studies report successful regulation and only limited evidence of improvements in task performance [145, 235]. While this may be disheartening at first glance, this growing body of research gives reason to believe that, in principle, behavioural performance can be improved with rt-fMRI-nf, even with relatively short training protocols. Possibly, the brain processes that were successfully regulated in this (Chapter 4) and other experiments are not the only ones responsible for influencing task performance. A more comprehensive approach of rt-fMRI-nf training may be needed to obtain stronger effects on task performance.

Furthermore, it is possible that the lack of substantial behavioural effects may relate to the *performance effectiveness* prediction of ACT. This proposes that task performance is often maintained in anxious individuals albeit with reduced *processing efficiency*, i.e. the quality of performance relative to use of processing or cognitive resources. Several studies have

shown increased DLPFC activity in people with HTA without concomitant improvements in *performance effectiveness* (i.e. *processing inefficiency*; [13-15]). Thus, increased DLPFC-ACC functional activity and connectivity, seen after rt-fMRI-nf training in the EG, may have improved attentional network *processing efficiency*, leading to a reduction anxiety levels (Chapters 4 and 6), but without a demonstrable effect on *performance effectiveness* in attentional control tasks.

As a limitation of these results, it is important to recognise that this study may not have produced large enough effects in task performance. Results of previous studies comparing HTA and LTA groups on performance in the colour Stroop task for instance have varied between small to medium effect sizes [13, 216] and this study was only powered to detect medium to large effect sizes. A significant small effect was detected in the EPT; however, studies assessing changes in dot-probe-based tasks pre- and post-attentional bias modification have yielded medium to large effect sizes, magnitudes not comparable to the present finding [224].

Future studies would need to recruit larger samples, while it may also be of value to examine changes in brain activity during attentional control to better understand *processing efficiency* versus *performance effectiveness*.

5.4.1. *Conclusions*

In conclusion, DLPFC-ACC functional connectivity-based rt-fMRI-nf had very limited effects on behavioural performance. There were no significant changes in inhibition or sustained attention that could be attributed to rt-fMRI-nf training. Effects on attentional bias were small, however,

supporting the notion of improved attentional control post-rt-fMRI-nf. These results may reflect relatively small effect sizes that this study was not powered to detect. However, speculative rt-fMRI-nf may have led to improvements in *processing efficiency*, which would not be apparent in task performance.

6. Effects of DLPFC-ACC Functional Connectivity-Based rt-fMRI-nf on Wider RSFC

6.1. Introduction

There has been a shift in neuroscience away from evaluating localised increases and decreases of brain activation in individual regions toward a wider perspective of examining functional networks [35]. These networks are characterised by synchronous activation and deactivation of structurally distinct brain regions, as studied using predominantly, but not exclusively, low frequency correlations in activation to establish RSFC. Networks measured with RSFC also show structural connectivity in the brain [239, 240] and specific alterations in RSFC have been associated with several different psychopathologies [41].

A most basic distinction has been made between a non-specific task positive or EMN, and an anticorrelated task negative network or DMN [35, 42]. More detailed distinctions between functional networks within the EMN have been made and associated with specific brain functions (e.g., [241]), in particular the FPN and the CON are described as attentional control networks within the EMN. The DMN is a network of regions including the Posterior Cingulate Cortex (PCC), Medial PFC and Angular Gyrus. Activation in the DMN has been associated with emotional regulation [36], mind-wandering [43] and attentional lapses [44]. DMN activation is anti-correlated with activation in attentional control networks [35] and failure to sufficiently deactivate the DMN can interfere with performance in cognitive tasks [44, 78]. The CON or salience network

includes the ACC and anterior insula and is important for error monitoring. The CON recruits both the FPN and the DMN for effective task processing [242]. The FPN is also known as executive control network and is often associated with top-down attentional control. Important regions or hubs in the FPN are the DLPFC and intraparietal sulcus.

Functional connectivity studies report dysconnectivity in attentional control networks in people with HTA, specifically between the DLPFC and the ACC [34, 71], hubs of the FPN and CON respectively [36]. Such dysconnectivity could underlie the inefficient allocation of neural resources in people with anxiety as the ACC is thought to be important for 'reactive' or 'compensatory' processes [37] that update the DLPFC when increased attentional control is required [79, 80]. Altered RSFC within the DMN and EMNs have been associated with anxiety disorders and also trait anxiety [36, 41, 75, 76]. In addition, interactions between the DMN and attentional control networks are reduced in anxiety [64].

Given the role of functional networks and their interactions in psychopathology and cognitive function, RSFC has been used as a pre- and post-training measure to determine successful neuromodulation in rt-fMRI-nf studies. Altered functional connectivity after rt-fMRI-nf-training has been reported in several studies during task (e.g., [113, 145]) and rest (e.g., [104, 141, 162, 163, 243]).

Furthermore, changes in RSFC have been associated with decreases in self-reported symptom severity in patients after rt-fMRI-nf (e.g., in depression [163] and auditory verbal hallucinations [243]). Interestingly

rt-fMRI-nf can affect wider RSFC, even when feedback is limited to a single target region (e.g., [141, 162]). Thus, changes in functional connectivity during rest are an especially important outcome measure for rt-fMRI-nf training protocols, as this allows researchers to investigate changes in network dynamics that are independent of self-regulation effort [155].

In this study, participants with HTA underwent rt-fMRI-nf targeting DLPFC-ACC functional connectivity. The current understanding of the exact mechanisms resulting in wider network changes after rt-fMRI-nf-training is limited, hence this study was considered somewhat explorative. Nevertheless, based on the importance of interactions between attentional control networks and DMN in attentional control tasks [44, 78, 242], and that these interactions are thought to be altered in anxious individuals [36, 64], it was hypothesised that DLPFC-ACC functional connectivity-based rt-fMRI-nf would alter RSFC in and between DMN and attentional control networks; specifically, that the anticorrelation between these networks would increase.

6.2. **Methods**

The study sample, experimental design and rt-fMRI-nf protocol used in this experiment are described in detail in Chapter 3. To assess the effects of rt-fMRI-nf training on RSFC, a resting-state fMRI scan was performed at pre- and post-rt-fMRI-nf training time points in 28 participants n = 28 (EG = 13, CG = 15).

6.2.1. *Data Acquisition*

Functional images during the 10 minute resting-state scan were acquired using a full-brain, anterior-to-posterior, T2* weighted, BOLD-sensitive gradient echo planar sequence with the following parameters: TR/TE/flip angle = 2 s/40 ms/70°, field of view 192 mm × 192 mm and slice thickness of 4 mm giving a voxel size of 3 mm × 3 mm × 4 mm and whole brain coverage of 28 interleaved slices. Three hundred volumes were collected while participants were instructed to *'stay awake and relax while keeping your eyes closed for the duration of the scan'*.

6.2.2. *Resting State Analysis*

Resting State fMRI data was analysed using MELODIC (FMRI Expert Analysis Tool) Version 3.14. Resting-state data was not available in two participants, hence the sample size was n = 28 (EG = 13, CG = 15).

During pre-processing, registration to high-resolution structural and/or standard space images was carried out using FLIRT [244, 245]. Registration from high resolution structural to standard space was then further refined using FNIRT nonlinear registration [246, 247]. The following pre-processing pipeline was applied; motion correction using MCFLIRT [245], non-brain removal using BET [248], spatial smoothing using a Gaussian kernel of FWHM 6.0 mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor; high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 50 s). Time-series statistical analysis was carried out using FILM with local autocorrelation correction [249]. Probabilistic

Independent Component Analysis (ICA; [250]) was then applied to the pre-processed data. The resulting single subject components were manually classified as either meaningful components or noise components [251] to remove artefacts from the data. Further FAST [252] segmentation was used to identify tissue classes at subject level and WM and CSF was regressed from the data.

Pre-processed data that had been cleared of artefacts were subsequently submitted to higher level group analysis using multi-session temporal concatenation in MELODIC with an a priori defined number of 15 output components. The resulting components were classified manually and by correlation with reference maps of validated connectivity networks [253]. As this study specifically focussed on network interactions between the DMN and attentional control networks (i.e. FPN, CON), suitable components were analysed and tested for significance. Remaining components were discarded. The spatial maps from the group-average were used to generate subject specific versions of the spatial maps and associated time series using dual regression [250, 254]. These were then tested for a time x group interaction using randomise non-parametric permutation testing (5000 permutations) with threshold-free cluster enhancement [255].

6.3. **Results**

From the 15 components derived in a group ICA, independent component 4 was selected based on an a priori hypothesis for testing group differences between pre- and post-rt-fMRI-nf training (*Figure 16*A) in attentional

control and DMNs. This component explained 7.03% of variance in the dataset, showing positive RSFC in ACC (peak x/y/z = 4/14/28) and bilateral Anterior Insula (left peak x/y/z = -34/4/0; right peak x/y/z = 36/2/0), resembling the topological structure of the CON. Independent component 4 also shows positive RSFC in the bilateral Inferior PFC (left peak x/y/z = -44/30/10; right peak x/y/z = 46/32/4), regions within the FPN, and negative RSFC in regions known to be involved in the DMN i.e. bilateral Angular Gyrus (left peak x/y/z = -44/-62/40; right peak x/y/z = 44/-62/44), bilateral Superior Frontal Gyrus (left peak x/y/z = -18/24/48; right peak x/y/z = 20/26/48) and PCC (peak x/y/z = -2/-44/28) [36, 253, 256]. Testing for the effect of rt-fMRI-nf pre- and post-training (post > pre) there was significantly increased RSFC in the bilateral PCC (peak x/y/z = 0/-24/38, t = 5.55, p = .025, Figure 16B), in participants in the EG compared to participants in the CG between pre- and post-rt-fMRI-nf training.

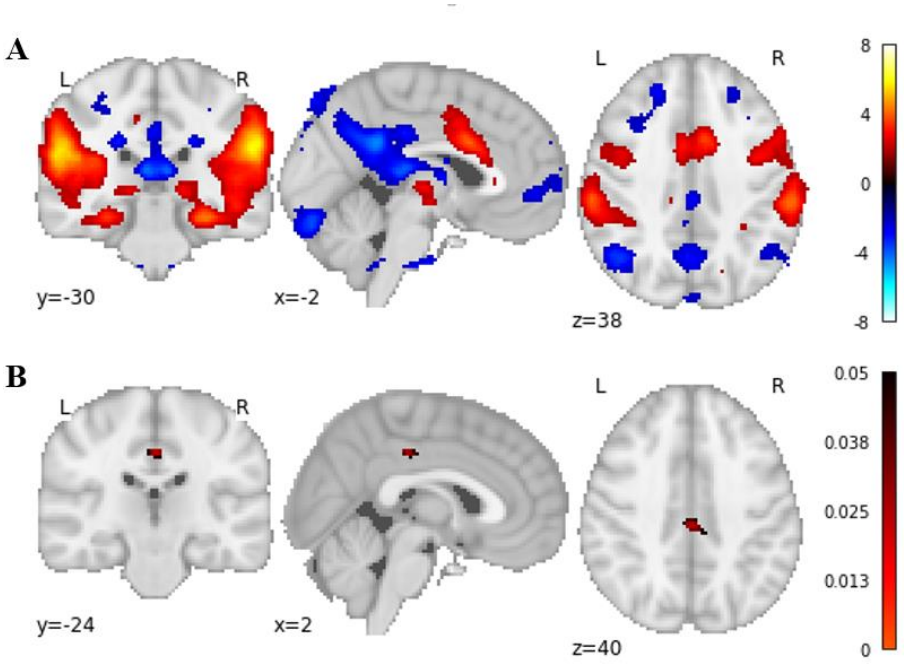

Figure 18. (A) Z-map for selected component 4 based on group ICA analysis showing RSFC in CON, FPN and DMN regions. (B) Increased RSFC in EG pre vs. post-rt-fMRI-nf training in the PCC (p-map).

6.4. Discussion

This experiment used connectivity-based rt-fMRI-nf training, targeting DLPFC-ACC functional connectivity, to examine the effects on wider RSFC in attentional control networks in HTA individuals. An independent component was identified that displayed the previously reported relationship between CON, FPN and DMN [242, 256, 257], networks known to be important for attentional control, where the CON modulates DMN activation. Further, the relationship between these functional networks is altered in HTA [36, 64]; hence it was hypothesised, that rt-fMRI-nf training may improve RSFC between these networks.

Our analysis of RSFC data showed that post-rt-fMRI-nf training, relative to the CG, the EG groups had increased RSFC in the PCC, a main hub in

the DMN [258]. Anxiety is thought to be associated with decreased functioning in DMN [36] that can effect emotional regulation and interactions with FPN during cognitive tasks and regulation [259]. Furthermore, recent fMRI research have shown that worry, a cognitive component of trait anxiety [260], and mind wandering both involve the DMN [43], and that anxiety and worry are associated with altered DMN activation [75]. These findings may indicate increased decoupling between DMN activation and attentional control networks, thus a normalization of network interactions that are important for cognitive processing [44, 78].

Whilst a range of functions have been ascribed to the PCC, Pearson and colleagues [261] propose a broader view of the PPC being a key node in the DMN for adapting behaviour in changing environments. In terms of attentional control, the PCC is described as a hub mediating interactions between the ACC and DLPC. Thereby the ACC is involved in monitoring the need for behavioural change and the DLPFC is a major site for executive control [261]. Similarly the PCC has been implicated in attentional control and modulating the interaction between DMN and attentional control networks [262, 263]. However, other brain regions may also mediate these network interactions [264]. A recent study to address the relationship between DMN activation and behavioural performance report that the degree of connectedness of the PCC with other regions predicted performance in an attention task [263]. In line with this, Weissman and colleagues [44] have shown that less efficient stimulus processing during attentional lapses is characterised by less deactivation in the DMN, particularly the PCC. Failure to deactivate the PCC during

attentional task may result in less efficient attentional control. Increased RSFC in this area, brought about by rt-fMRI-nf training, may facilitate more efficient interactions between DMN and attentional control networks.

While there is some evidence of altered activation of the DMN in people with HTA during rest (e.g., [75]), there is not much evidence on how RSFC in the DMN is altered in HTA. Nevertheless, Modi and colleagues [265] performed a study on the relationship of trait anxiety and RSFC in a number of functional networks. In this study, individuals with HTA, compared to LTA, exhibited reduced functional connectivity in the DMN, specifically the PCC. In context of this research, these findings could be interpreted as "normalization" of RSFC in the EG.

These results add to a growing body of literature documenting changes in RSFC after rt-fMRI-nf, specifically altered network interactions with the DMN. A recent study investigating the effects of rt-fMRI-nf training on areas within the left language network, reports increased coupling between this language network and the DMN specific to the population and direction of rt-fMRI-nf [243]. Similarly the coupling between DMN and motor-visuospatial network has been increased after rt-fMRI-nf on the functional connectivity between nodes of both networks [104]. These studies have used network analysis with predefined ROIs as well as more exploratory whole-brain approaches. Similarly in the current study, RSFC was examined at a whole-brain level, while the analysis was focused on the DMN and attentional control networks, which were modulated in the

rt-fMRI-nf task. The finding of increased RSFC in a component including both DMN and attentional control networks strengthens the evidence for the use of rt-fMRI-nf to modulate network dynamics beyond the rt-fMRI-nf target regions.

It is remarkable that along with other rt-fMRI-nf studies, this research demonstrated the wider effects of rt-fMRI-nf training on functional networks beyond the targeted brain regions, particular within the DMN. It is currently not understood which precise mechanisms lead to these changes in network dynamics. Having demonstrated the feasibility of rt-fMRI-nf training of activation in single ROIs and functional connectivity between two areas to alter whole brain mechanisms connected to the rt-fMRI-nf targets opens many pathways for clinical interventions and for future research.

6.4.1. *Limitations and Future Directions*

In using an ICA approach to evaluate RSFC, this study has some limitations regarding the interpretation of findings being somewhat speculative. However, ICA is a powerful whole-brain approach to explore changes in RSFC in specific networks, it is data-driven and does not test changes in connectivity between specified ROIs. In contrast, seed-based functional connectivity analyses are correlation-based measure of functional connectivity to a predefined ROI. Seed-based approaches have been used frequently to evaluate RSFC after rt-fMRI-nf (e.g., [141]), they are easier to interpret and test specific connections between areas. However, these approaches are limited by the selection of a pre-defined

ROI and are therefore not always the most suitable approach when investigating whole-brain changes in network dynamics [155].

Furthermore, these findings must be considered in the context of the relatively limited understanding of the relationship between cognitive function and network dynamics between DMN and attentional control networks. There is a general consensus that the DMN functioning is decreased in anxiety; however, not all evidence is consistent with this prediction [36, 41]. In addition the PCC is a heterogeneous area whereas different proportions may have different functional roles in modulating network dynamics [262], this needs to be explored further in future work.

Future work could further explore the role of network interactions during task and rest periods, and how different nodes function in the transitions between these states. Furthermore, more basic research is needed to better understand the underlying processes of altered RSFC brought about by rt-fMRI-nf training. Previous work has shown effects of rt-fMRI-nf on RSFC [104, 141, 162, 163, 243], but few studies address the underlying processed evoking these changes are unclear. Speculatively, rt-fMRI-nf may lead to changes in neuroplasticity by strengthening important connections and weakening unimportant ones [145, 155]. Investigating changes in functional connectivity as a function of the amount of rt-fMRI-nf may be a viable approach to further explore this hypothesis.

6.4.2. *Conclusion*

To conclude, these results show increased RSFC in the PCC, a region in the DMN after up-regulating DLPFC-ACC functional connectivity using

rt-fMRI-nf training. This effect was observed specifically in the EG, suggesting a causal link between rt-fMRI-nf training and increased RSFC. The exact mechanisms behind this finding are not clear, however. Speculatively, the PCC may be acting as a hub within the DMN and increased RSFC in this region may represent increased efficiency in network interactions between the DMN and attentional control networks such as FPN and CON. More research is required to better understand the role of interactions between functional networks in the brain during rest but also during task processing.

7. Conclusions

7.1. Summary of Hypotheses and Findings

This body of work aimed to investigate the neural processes underlying impaired attentional control in people with HTA, specifically focussing on neural *processing efficiency* and *performance effectiveness*. A further aim was to investigate the feasibility of functional connectivity-based rt-fMRI-nf training to restore efficient processing, increase performance in attentional control tasks and reduce anxiety levels.

Firstly, a combined ^{1}H-MRS-fMRI study examining the relationship between trait anxiety, DLPFC activation during an attentional control task (Stroop task), and PFC Glu levels was conducted (Chapter 2). Consistent with the a priori hypothesis, participants in the HTA group showed reduced *performance effectiveness* during an attentional control task when task demands were high. However, trait anxiety had no effect on brain activation during an attentional control task or resting-state PFC Glu levels. This was contrary to previous findings that reported a relationship between trait anxiety and increased task-related DLPFC activation [13] and increased Glu concentrations [96]. Nevertheless, there was a significant interaction between PFC Glu levels, trait anxiety and left DLPFC activation during incongruent task trials. In the LTA group there was a positive relationship between excitatory neurotransmission and task-related activation in the DLPFC, which was absent in the HTA group. The relationship between brain activation measured with fMRI and metabolite levels is a relatively unexplored area of research. However, the observed

results may have elucidated further details about ineffective task processing in people with HTA (Chapter 2).

Secondly, a series of studies utilising a randomised controlled design and rt-fMRI-nf to modulate DLPFC-ACC functional connectivity in HTA individuals was used to investigate the effects of DLPFC-ACC functional connectivity-based rt-fMRI-nf on anxiety levels, brain activation and connectivity in the target regions, attentional control performance, and RSFC in DMN and attentional control networks (Chapters 3-6). Founded on previous studies using rt-fMRI-nf training, a novel rt-fMRI-nf setup was specifically developed for this research project, which allowed participants to monitor functional connectivity between the left DLPFC and the bilateral ACC in real-time. Rt-fMRI-nf parameters were customised to each participants' activation in and connectivity between the left DLPFC and bilateral ACC, parameters acquired during a functional localiser scan based on the Stroop task. HTA participants were pseudo-randomly allocated to an EG (receiving veridical feedback) and a CG (receiving sham feedback) to provide a between–subjects control. Furthermore, a range of offline tasks, psychometric and MRI measures were acquired at pre- and post-rt-fMRI-nf, time points, providing a within-subjects control. This approach ensured a statistically powerful design and limited the influence of confounding factors (Chapter 3).

A primary aim of this series of studies was to establish the feasibility of functional connectivity-based rt-fMRI-nf training to alter activation and functional connectivity in the left DLPFC and bilateral ACC. The study

reported in Chapter 4 showed that both activation and connectivity in the left DLPFC and bilateral ACC were predominantly increased after rt-fMRI-nf training and that any group difference post-rt-fMRI-nf were driven by changes in the EG and not by changes in the CG. Furthermore, the EG exhibited reduced anxiety levels post-rt-fMRI-nf training that were associated with increased activation in the target regions during rt-fMRI-nf training. No other psychometric measures (i.e. depression and stress) were significantly altered post- rt-fMRI-nf training (Chapter 4).

Based on the previous research reporting an association between DLPFC-ACC functional connectivity and reduced *performance effectiveness* in HTA individuals [13, 34], it was hypothesised here that rt-fMRI-nf training targeting connectivity between these regions would improve performance during attentional control tasks (both with and without threat-related stimuli). Whilst there were some indications of reduced bias towards emotionally salient stimuli post-rt-fMRI-nf, there appeared to be very limited effects on attentional control overall. Possible reasons for the absence of this effect have been discussed (Chapter 5).

Finally, network effects of rt-fMRI-nf training were examined using RSFC with a particular focus on the DMN and attentional control networks, which have previously been associated with impaired attentional control in HTA individuals [36]. An independent component representing RSFC in regions of FPN, CON and the anti-correlated DMN was selected based on these a priori considerations. Within this component, the EG showed increased RSFC in the PCC post-rt-fMRI-nf. The PCC is a main hub of

the DMN and has been implicated in important interactions between DMN and attentional control networks – specifically FPN and CON. Rt-fMRI-nf training may have increased these network interactions between the DMN and attentional control networks (Chapter 6).

In sum, two lines of research have been conducted; the results from a combined ^{1}H-MRS-fMRI study indicate reduced *performance effectiveness* in HTA participants, when task demands are high, that may be related to an altered relationship between neural processing and excitatory neurotransmission. In addition, a rt-fMRI-nf experiment in HTA participants, revealed that veridical rt-fMRI-nf training of DLPFC-ACC functional connectivity increased brain activation and connectivity in target regions, reduced anxiety levels and increased RSFC.

7.2. **Discussion**

7.2.1. *Implications for Attentional Control Theory*

ACT outlines how HTA can affect both *performance effectiveness* and *processing efficiency* during attentional control tasks [7]. The theory postulates that high levels of worry, which are typical in people with HTA, take up cognitive resources, hence impairing attentional control. Performance is especially predicted to be impaired, when task demands on the inhibition and shifting function are high. Furthermore, reduced attentional control in HTA individuals is assumed to be domain-general (i.e. with and without threatening stimuli). In a combined fMRI and ^{1}H-MRS study (Chapter 2), the HTA group showed reduced *performance effectiveness* (increased ER) during an attentional control task only for

incongruent task trials, which require greater attentional control, whilst performance during low cognitive demand trials was unimpaired. Basten and colleagues [13] reported a similar finding, where participants with HTA only showed impaired performance during a task condition with high cognitive demands. Furthermore, the HTA group generally showed slower RTs compared to the LTA group. These findings are consistent with reduced *performance effectiveness* and *processing efficiency* predicted by ACT [7]. Notably, the attentional control task used here, and by Basten at al. [13] did not include a threat component.

In the rt-fMRI-nf experiment, only participants with HTA were recruited, hence comparison of task performance between groups with LTA and HTA was not possible. However, although anxiety levels were reduced post-rt-fMRI-nf training, there was no consistent relationship between reduced anxiety levels post-rt-fMRI-nf and changes in attentional control task performance. It is unclear why this study yielded no substantial effects on task performance, possible reasons may be small effect sizes or improvements in *processing efficiency* post-rt-fMRI-nf, which did not translate into increased *performance effectiveness* (Chapter 5). Previous behavioural studies have shown that whilst anxiety can effect *processing efficiency*, *performance effectiveness* is not always affected [17].

While deficits in attentional control have also been associated with other psychopathologies, in particular with depression [266, 267], ACT states that impoverished attentional control in HTA is due to processes that are specific to anxiety. Similarly, in the findings reported here, rt-fMRI-nf

training of DLPFC-ACC functional connectivity specifically reduced anxiety, but did not affect depression or stress levels (Chapter 4). This finding suggests that the mechanisms modified by the functional connectivity-based rt-fMRI-nf training may be specific to anxiety.

On a neural level, ACT does not provide a comprehensive framework of the processes underlying impaired attentional control and its neurocognitive predictions do not go beyond loosely localising attentional systems to brain areas [7]. Eysenck and colleagues [7] almost exclusively base their theory on findings from behavioural studies, which is unsurprising considering the dearth of relevant cognitive neuroscience research prior to 2007. However, a number of empirical neuroimaging studies have provided more precise information about the functional brain networks and neurofunctional processes that are relevant to ACT (see Chapter 1.1.).

These findings form the basis for the development of a neurocognitive framework that supports the predictions of ACT. Specifically, three attentional control networks appear to be important for the attentional processes relevant to ACT; FPN, CON and VAN. In brief, the FPN has been recognised as a neural derivative of goal-directed attention, the CON as a system important for error monitoring and more broadly reactive attentional control: respectively, the VAN is a key network for stimulus-driven attentional control [11, 36]. In addition, the DMN, although not strictly an attentional control network, has also been implicated in impaired attentional control. The DMN has been shown to be altered in

HTA *and* to interact with attentional control networks during task processing [242]. Chapter 1.1. outlines how connectivity between these networks and between core hubs of these networks is important for optimal cognitive processing. Specifically, DLPFC-ACC functional connectivity is shown to be important in ensuring the balance between goal-directed and stimulus-driven attention. ACT predicts that this balance is disrupted in individuals with HTA, contributing to impaired attentional control.

A key concept of ACT is that of *processing (in)efficiency*. In previous literature *processing efficiency* has been defined as the ratio between allocation of effort/energy and behavioural output. Cognitive neuroscience, provides the potential to explore the full complexity of *processing* efficiency, because of the very rich data that can be collected, in contrast to behavioural measures. Neurally, *processing inefficiency* is conceptualised on different levels throughout this thesis, as different methods were applied and different measurements were taken. Regarding neural efficiency in the context of brain activation as measured with fMRI; increased brain activation in participants with HTA that is concomitant with equivalent behavioural performance (relative to participants with LTA), or, equivalent activation with reduced behavioural performance, can both be interpreted as inefficient neural processing (Chapter 2). Furthermore, an interpretation of neural efficiency was applied to the observed altered relationship between neurotransmitter levels and task-relevant brain activation. Participants with LTA and HTA had comparable PFC Glu levels and task-related brain activation; however, only in the LTA group were PFC Glu levels related to brain activation during an attentional

control task (Chapter 2). This was interpreted as reduced *processing efficiency* in HTA, as neurotransmitter levels did not appear to affect task-related activation. Finally, the term neural efficiency was used in the context of functional connectivity and network dynamics. Increased functional connectivity between DLPFC and ACC was viewed as a pattern of increased neural efficiency based on literature suggesting that DLPFC-ACC dysconnectivity may contribute to inefficient processing in people with HTA (e.g., [13, 34, 71]; Chapter 4). Furthermore, increased RSFC in the PCC within a component containing elements of DMN and attentional control networks was also interpreted as increased neural efficiency (Chapter 6). This was based on previous literature showing a connection between dysfunctional DMN and reduced behavioural performance in cognitive tasks [44, 78]. This is a more speculative interpretation of neural inefficiency and more research is needed to understand the functional consequences of reduced or increased RSFC.

Whilst the findings of this thesis are broadly in line with previous neuroimaging studies investigating the effects of anxiety on attentional control and its neural correlates, the current findings also offer some new insights into the mechanisms that may contribute to impaired attentional control in anxious individuals. The combined ^{1}H-MRS-fMRI study reported in Chapter 2 highlights the key role of PFC Glu levels in *processing efficiency*. The positive relationship between PFC Glu levels – an excitatory neurotransmitter - and task-related brain activation in the DLPFC observed in the LTA group was absent in the HTA group. This may be one mechanism through which trait anxiety can impair efficient

neural processing, i.e. via reduced capacity for energy turnover. To speculate, in the HTA a greater proportion of PFC Glu may have been employed for anxiety-related non-task processing (i.e. worry). Despite this novel finding, DLPFC activation did not differ between anxiety groups, contrary to previous research (e.g., [13-15]); neither did the groups differ on PFC Glu levels, which had been shown in the literature [96].

In a connectivity-based rt-fMRI-nf experiment (Chapter 4), a positive relationship between brain activation in DLPFC and ACC during rt-fMRI-nf regulation and reduced anxiety levels (post-rt-fMRI-nf training) was observed. Both the DLPFC and ACC show inefficient activation in HTA participants (e.g., [15, 70, 71]), and while more research is needed to better understand the exact reasons for this, rt-fMRI-nf training of DLPFC-ACC functional connectivity may have led to increased neural efficiency in these areas that subsequently reduced anxiety levels. This is an important finding for ACT, as previous work has been predominantly correlational. This experiment strengthens the case of a bi-directional relationship between HTA and impaired attentional control.

Finally, analysis of RSFC pre- and post-rt-fMRI-nf training (Chapter 6), showed increased RSFC in the PCC – a main hub of the DMN and a key area mediating interactions between the DMN and DLPFC and ACC [261]. There is emerging evidence that interactions between DMN and attentional control networks are important for effective and efficient task processing [44, 78]. Additionally, the interaction between DMN, FPN and CON have been shown to be important for effective attentional control

[242]. Up-regulating functional connectivity between DLPFC and ACC, hubs of the FPN and CON respectively may have had a wider effect on network dynamics including the DMN, potentially leading to increased *processing efficiency* in the EG.

Importantly, the results from Chapters 4 and 6 were not accompanied by the hypothesised improvements in *performance effectiveness* in attentional tasks (Chapter 5). While there were some reductions in attentional bias to emotionally salient stimuli, these effects were small and there were no further effects on other attentional control tasks. It can only be speculed as to whether changes in neural activation and connectivity led to increased *processing efficiency* without having more conclusive insight into changes in performance and changes in task-related brain activation. As there was no LTA control it is unclear whether the sample showed reduced *performance effectiveness* pre-rt-fMRI-nf in any of the tasks used.

In sum, this work has important implications for ACT in terms of understanding the neural mechanisms that underlie reduced *processing efficiency*. Traditional theory and research in the field of attentional control and anxiety were primarily based on behavioural findings, which are limited in exploring the complexities of what is involved in *processing inefficiency* in HTA individuals. This cognitive neuroscience research helps to better understand the differences in brain activation, connectivity, neurotransmission, and network dynamics between HTA and LTA individuals. Importantly, both lines of research presented in this thesis did not solely focus on activation or deactivation of isolated brain regions, but

took a network approach of brain function. Furthermore, rt-fMRI-nf enabled this research to employ an experimental design to investigate predictions of ACT, which is predominantly based on correlational evidence. Therefore, this work goes beyond existing findings by manipulating and measuring aspects of *processing efficiency* to study the influence on anxiety levels. The findings in this experiment strengthen the notion that the relationship between HTA and impaired attentional control is indeed bi-directional. In addition, both lines of research focused on domain-general neural processes of attentional control, supporting the theoretical framework of ACT.

There is important work to be done in the future to refine the understanding of which neural processes and network dynamics are especially involved in reduced attentional control and neural inefficiency in HTA, as described by ACT. Rt-fMRI-nf training may prove a useful tool in furthering the understanding of *processing efficiency* as it may be used to normalise processes in the brain that show abnormal neural processing in HTA even when *performance effectiveness* is not impaired.

7.2.2. *Implications for Connectivity-Based rt-fMRI-nf*

Over recent years there has been a growing number of rt-MRI-nf studies applied across clinical and non-clinical populations [111]. However, to this point there is a relatively small number of studies using connectivity-based rt-fMRI-nf. Rt-fMRI-nf based on measures of functional or effective connectivity consider cognitive processes on a network level rather than focusing on activation in individual brain regions, a recent and important

shift in cognitive neuroscience (e.g., [268-270]). Traditional cognitive neuroscience is limited in this respect and neurocognitive models have outgrown this rather reductive approach of mainly focussing on the specialised functions of individual brain regions. Hence, connectivity-based rt-fMRI-nf is more suitable to modify complex cognitive processes as described by ACT.

Researchers have applied different measures of connectivity (i.e. functional and effective connectivity) to rt-fMRI-nf training protocols with some positive initial findings (e.g., [104, 105, 122, 124, 128]). Connectivity-based rt-fMRI-nf training has opened many pathways for the use of rt-fMRI-nf in clinical applications and cognitive enhancement. Equally as important, connectivity-based rt-fMRI-nf has the potential to causally test sophisticated neurocognitive models and processes compared to rt-fMRI-nf studies targeting single neural regions. Functions related to connectivity and network interactions are at the core of many neurocognitive models, not least of ACT.

Specifically, rt-fMRI-nf based on functional connectivity (i.e. correlational measures between brain regions) is promising as it is relatively easy to implement and less computationally demanding compared to other techniques (e.g., compared to DCM-based rt-fMRI-nf; [123]). Furthermore, recent advances in open science practice are leading to a wider availability of expertise and resources, enabling more researchers to implement these complex methods.

Currently, there is no one established protocol to administer functional connectivity-based rt-fMRI-nf. The few studies conducted in this area have all employed different techniques. Megumi and colleagues [104] provided intermittent feedback based on the functional connectivity between two areas that were anatomically defined. Another study by Zhao and colleagues [122] used a sliding time window and thus provided a measure of dynamic functional connectivity as the partial correlation between two brain areas, defined with a functional localiser, while accounting for white matter. This methodology is in principle similar to the setup presented here; however, in their study, Zhao and colleagues [122] did not provide sufficient information for replication of their design. For example, length of the sliding window, the size of the ROIs used and the location of the WM control region were not specified.

Here an approach to functional connectivity-based rt-fMRI-nf is presented that is highly standardised, yet customises feedback to each individual participant according to their brain activation and connectivity, established during a functional localiser task. Importantly, there are many shortcomings in experimental design that have made rt-fMRI-nf studies a target of criticism [111]. The work presented in this thesis has tried to address some of these criticisms. First, participants were pseudo-randomised to either experimental or control group in a single-blind design. Second, employing an external control group, pre- and post-comparisons on all measurements were conducted, hence providing an additional internal control. Next, the two ROIs for regulation were defined based on a functional localiser task and the same method of localisation

was employed in both experimental groups. A third ROI was defined to account for general brain activation and global scanning effects and size and location of all ROIs were reported. Finally, a series of pre- and post-measures were administered to establish the effects of rt-fMRI-nf training on brain activation and connectivity, cognitive performance and psychometric measures.

The methodology used for the rt-fMRI-nf setup has been reported in much detail and custom scripts used have been made available (Appendix 5) to allow for replication of this study as well as for adapting the general setup to other research questions.

While functional connectivity-based rt-fMRI-nf may be easier to implement and interpret than DCM-based rt-fMRI-nf, no study has attempted to provide a direct comparison of the efficacy of both methods. DCM-based rt-fMRI-nf arguably allows for more complex neural mechanisms to be trained, while the ability of participants to monitor and develop strategies to manipulate neural processes may be limited.

7.2.3. *Implications for Modulating Attentional Control in People with High Trait Anxiety*

Traditional interventions for modifying attentional control in people with HTA are usually designed to reduce attentional bias to threat (e.g., [222-224]). Whilst some of these interventions have been shown to significantly reduce attentional bias and anxiety levels in various anxious populations, generally the results of these studies have been mixed [222-224]. Attentional bias modification interventions are also limited, as they do not

generally address impairments in attentional control in the absence of threat-related stimuli [6, 13-15]. Furthermore, cognitive training alone, while improving *performance effectiveness*, may be insufficient in changing the underlying deficiencies in *processing inefficiency*.

Rt-fMRI-nf targeting neurocognitive processes that are altered in HTA addresses some of the limitations associated with traditional attentional bias modification interventions. As already discussed, DLPFC-ACC functional connectivity has been shown to be reduced in HTA contributing to inefficient task processing in attentional control tasks, independently of whether threat-related stimuli are present or not (e.g., [13, 34]). Furthermore, while it was hypothesised that this rt-fMRI-nf study would improve *performance effectiveness*, the rt-fMRI-nf training primarily targets a mechanism related to *processing efficiency*. The findings presented here do not show comparable changes in behavioural performance as have been reported in attentional bias modification research. Nevertheless, significant rt-fMRI-nf training effects on anxiety levels and brain activation and connectivity have been shown, and as already discussed, these may be reflective of improvements in *processing efficiency*. These improvements may transfer to improved *performance effectiveness* in different contexts, although this claim would need to be tested in future work.

7.3. Limitations and Strengths

The research presented in this thesis has produced a series of novel findings that contribute to the scientific understanding of ACT and the use

of functional connectivity-based rt-fMRI-nf in HTA individuals. This is the first reporting of differences in the interaction of PFC Glu levels and task-related brain activation between HTA and LTA participants. Furthermore, this thesis also makes a significant contribution to methodology for functional connectivity-based rt-fMRI-nf.

First, the details of the rt-fMRI-nf methodology have been reported with considerably more detail compared to previous studies, with the aim of making the functional connectivity-based protocol that was used reproducible. Additionally, custom scripts have been written in the widely used and open source programming language python and made available in Appendix 5 of this thesis and at Open Science Framework DOI 10.17605/OSF.IO/SYNEU.

Overall, this research constitutes an important advance in the use of functional connectivity-based rt-fMRI-nf, demonstrating that it is feasible to alter functional connectivity and affect behaviour (i.e. reduce anxiety levels).

Concerning Chapter 5, reporting the effects of rt-fMRI-nf training on cognitive control, there is some evidence from previous research that rt-fMRI-nf can be used for cognitive enhancement; however, effect sizes can be small [169]. In the present study, strong and/or consistent behavioural effects of rt-fMRI-nf were not observed. The theoretical reasons for this negative finding have already been discussed above. However, it cannot be ruled out that a lack of significant effects was due to insufficient power, meaning small effect sizes could not be detected.

Expanding on previous literature reporting changes in RSFC due to rt-fMRI-nf on a single target region [141, 162], the study reported in Chapter 6 documents increased RSFC in the PCC post-rt-fMRI-nf training. This exploratory finding, while needing confirmatory replication, is a meaningful addition to the understanding of how rt-fMRI-nf may work i.e. effecting connectivity changes in wider brain networks.

Furthermore, statistical rigor has been applied throughout data analyses reported in this thesis. This ensures reliability and validity of the findings. Data acquisition and analysis have been reported in much detail and stringent statistical thresholding has been applied throughout to avoid false positive findings and increase replicability [271].

Nevertheless, there are constraints due to sample size, which may limit the generalizability of these findings. Although power calculations showed that there was sufficient power to detect medium to large effects; larger multicentre studies with greater power to detect small effect sizes are needed to ensure more reliable and replicable outcomes [272].

Another potential limitation is that study participants were predominantly recruited from student populations. University students are considered to have marginally greater levels of trait anxiety in standardized norms [4]. Indeed, both samples displayed average trait anxiety levels that were slightly higher than published norms. Furthermore, it is to be expected that university students have higher than average IQ scores, which may have skewed effects on cognitive performance. Estimated IQ scores confirm that both samples scored above the national average [273]. This potential

limitation is shared by most other experiments in the field. It should also be noted that two of the 30 study participants were left-handed and these were both were in the EG. It is not clear if and how laterality may have affected the results.

It is also important to recognise the technical limitations associated with neuroimaging studies. Specifically fMRI and ^{1}H-MRS have several technical constraints. Firstly, in Chapter 2 resting-state ^{1}H-MRS was used to measure neurotransmitter concentrations and these Glu concentrations were then used in association with a measure of task-related brain activation. The underlying processes measured with resting ^{1}H-MRS and interactions with task-related activation are poorly understood. While the use of resting-state ^{1}H-MRS is common practice, research has shown that there are considerable differences between neurotransmitter concentrations between rest and task states, and that Glu levels dynamically measured during task may be a more accurate measure to capture task-related metabolism and understand the neural basis of cognitive processes [85, 214]. Measuring task-related metabolite levels with ^{1}H-MRS, although possible, is technically much more difficult. Thus the use of resting-state ^{1}H-MRS limits the interpretation of these findings and warrants replication including the measurement of task-related ^{1}H-MRS during an attentional control task.

Although, ^{1}H-MRS has been shown to produce reliable measurements of Glu levels [274], metabolite levels can depend on the tissue composition within the voxel (i.e. the amount of CSF, GMV and WMV; [192]).

Furthermore, inter-individual differences in GMV can influence these measurements [193]. Therefore strict statistical corrections were applied accounting for different tissue types within the PFC voxel. Additionally, as previous studies have used ratios relative to the Cr levels, without accounting for the composition of the voxel [87, 196], analysis with Cr corrected Glu are also reported.

Secondly, the fMRI signal is based on the paramagnetic effects of deoxygenated blood and thereby is an indirect measure of neural activation. While there is a general consensus that the signal measured with fMRI is proportional to neuronal activity, this assumption is dependent on a number of factors and there is a need to better understand the exact relationship between neural activity and the fMRI signal. In addition, fMRI is limited in its temporal resolution, as the hemodynamic response is considerably slower than the pace of neuronal firing [60, 275].

The low temporal resolution in fMRI presents specific challenges for *real-time* fMRI. Rt-fMRI-nf is only *real-time* regarding the changes in blood oxygenation, not regarding changes in neural firing. While participants are informed of this delay in the signal it is unclear how this impacts feedback learning. The signal delay may or may not present a major challenge in the participants' ability to find a suitable strategy to upregulate the feedback signal.

This research was primarily conducted within the framework of ACT. Section 7.2.1. of this chapter outlines how *processing efficiency*, a central concept in ACT could be understood on a neural level. However, using the

explanation of neural inefficiency to interpret findings in cognitive neuroscience has been criticised. Poldrack [215] argues that the concept of neural inefficiency is frequently ill-defined and the term has been used without sufficient explanation of the proposed underlying processes. Here the interpretation of neural inefficiency is founded on the theoretical framework of ACT. While in some contexts neural efficiency was defined as the ratio between cost (i.e. amount of brain activation) and behavioural outcome, neural efficiency in the context of functional connectivity or RSFC is more complex and this is acknowledged that some of the interpretations regarding processing and neural efficiency discussed here are speculative. However, the findings reported in this thesis provide a good basis for further investigation into how anxiety might affect neural and *processing efficiency*, i.e. activation, neurotransmission, connectivity and network interactions.

Some limitations also apply to the rt-fMRI-nf setup described in Chapter 3. Rt-fMRI-nf training based on functional connectivity using sliding-windowed correlation to compute the feedback signal is a very new technique and optimal parameters for its application have not been fully established. While sliding windowed correlation generally, has been identified as a suitable tool to provide feedback on functional connectivity [123], participants are fed back a composite score of their functional connectivity over the duration of the length of the sliding window. This requires the ability to maintain a strategy for a prolonged period of time and to evaluate the feedback signal according to its delay and how it was

derived. Therefore, functional connectivity-based rt-fMRI-nf may demand a considerable degree of cognitive resources from participants.

Furthermore, currently there is insufficient literature to determine the optimal window length for rt-fMRI-nf applications. There is a trade-off between using shorter time windows, which ensure immediacy of the feedback and may reduce the requirements for participants to maintain strategies for long periods of time, compared to longer time windows which may achieve a more reliable and stable signal [123]. In the rt-fMRI-nf study performed here, a relatively long time window was used (i.e. 20 s; Chapter 3), while one other study employing a sliding windowed approach showed successful regulation in participants using a sliding window of 7.5 s [122].

A further issue are the cognitive demands of self-regulation effort. Such an increase in cognitive demands may be a confounding factor in the interpretation of rt-fMRI-nf training effects. It is possible, that some of the effects of rt-fMRI-nf training may be due to self-regulation effort and its cognitive demands rather than true self-regulation of brain connectivity [109]. In a meta-analysis on the effect of rt-fMRI-nf regulation, self-regulation effort has been reported to increase activation in FPN, VAN and CON and decrease activation in DMN areas independently of the target region or direction of regulation [109]. This pattern is of similar topology as activation in the EMN and DMN during cognitive tasks [35, 42].

However, the results reported show distinct differences between the EG and the CG in a blind controlled setting. Throughout all fMRI analyses, a

clear interaction effect between group and time point was identified and the effects of rt-fMRI-nf training were specific to participants who received veridical rt-fMRI-nf rather than sham feedback (Chapter 4). Participants in the EG and the CG were unaware of which group they had been allocated to and all participants received the same instructions throughout. Therefore, it is expected, that participants in both groups would have devoted similar effort to self-regulate their brain connectivity. Hence, the effects of rt-fMRI-nf training on brain activation and connectivity cannot simply be explained by self-regulation effort. Using pre- and post-rt-fMRI-nf training resting-state scans further demonstrates that self-regulation had effects on brain activity beyond self-regulation effort.

In a critical review Thibault and colleagues [111] outline a number of issues in rt-fMRI-nf studies, including a lack of statistical comparisons to validate the successfulness of rt-fMRI-nf training. They propose the comparison of rt-fMRI-nf results to a baseline measure and compared to a control group. In this study, rt-fMRI-nf effects based on the interaction of group and time point were evaluated, providing an adequate control for confounding factors and ample statistical power to detect medium to large effect sizes.

In addition, the yoked feedback received by the control group ensured that there was no way for participants to deduct their group identity as both groups received the same visual input from the gauge interface. Is has been suggested that experience of successful regulation may have therapeutic

effects in itself [131, 132], hence yoked feedback in the control group was deemed most suitable to minimise this effect. Overall, the design of this experiment provided a strong external control to reduce the impact of confounding variables. Furthermore, although the rt-fMRI-nf protocol did not include a transfer run to examine if participants could continue successful regulation in the absence of feedback, a number of pre- and post- measures were applied to evaluate the efficacy of rt-fMRI-nf training, namely questionnaire measures, task measures and resting-state fMRI.

Some rt-fMRI-nf studies distinguish participants as learners and non-learners [113, 119, 276]. For instance, in a study by Scharnowski and colleagues [113, 276] four of eleven subjects were categorised as non-learners. Differentiating between learners and non-learners can add clarity to results as noise is reduced by removing participant variance that is not explained by rt-fMRI-nf training. Critically, this practice shrinks sample sizes and power in analysis and there is a danger of overestimating the efficacy of rt-fMRI-nf due to circularity in selecting a sub-sample of participants based on criteria that are dependent on major analysis outcomes (i.e. rt-fMRI-nf regulation success; [111]). For these reasons, this approach was not used in the current study.

Finally, while interview data has been collected on participants subjective experience of successful regulation and strategies used (Appendix 4), this data has not been recorded and analysed systematically. Comparing

motivation, self-efficacy and strategies used across groups would have allowed for important insights in this experiment.

In short, this research has produced novel findings, using rigorous study designs, which were reported in much detail. Rigid significance criteria and statistical thresholds were applied to all analyses and the research is framed in a strong theoretical basis provided by ACT. Nevertheless, the limitations of this research are discussed here, for example sample size, technical constraints and a need for more in depth understanding of underlying neural processes that warrant for future research.

7.4. Future Directions

In this thesis a range of novel findings have been presented from which a number of questions for future research can be derived.

First, the findings of this work should be used to inform a neurocogntivive account or framework of how trait anxiety affects attentional control. Whilst ACT is an influential and useful theoretical framework that provides a good foundation for cognitive predictions, it does not currently suggest detailed neurocognitive mechanisms through which anxiety can affect attentional control. Over the last 12 years, since the publication of ACT a large body of relevant cognitive neuroscience research has been conducted. Chapter 1.1. provides an overview of this empirical neuroimaging research on impaired attentional control in HTA, specifically the concept of impaired *processing efficiency* is addressed. The work conducted here could be the basis for the development of a

neurocognitive framework for ACT that incorporates empirical evidence from neuroimaging studies.

In conjunction with this, there is a need for more empirical neuroimaging research on anxiety and cognitive control, especially for multimodal neuroimaging studies, for example Hoffmann and colleagues [47], and Karch and colleagues [65], combining fMRI and EEG, or Falkenberg and colleagues [87] combining fMRI and ^{1}H-MRS. Research combining two or more neuroimaging techniques are of particular use when investigation the concept of *processing efficiency*. For instance the combination of fMRI and EEG delivers insight into temporal dynamics and a close approximation of neural activity with EEG, with the benefit of the greater spatial resolution and source localisation of fMRI. Furthermore, adding fMRI measurements to a ^{1}H-MRS study provides additional information on how neurotransmitter levels are utilised and translate into task-related brain activation, which may prove an important aspect of neural efficiency. In addition, future studies should empathise the importance of functional networks rather than individual brain regions, as this approach can be overly simplistic and misleading.

Notably, multimodal approaches have also been employed in rt-fMRI-nf studies. Zotev and colleagues [114] recently performed a rt-fMRI-nf study on PTSD patients in which EEG recordings were taken simultaneously to fMRI measurements during rt-fMRI-nf training. They cross-validated measures of altered functional connectivity between the two methods and while more research in this area is needed their findings suggest that their

intervention may also be effective using EEG-neurofeedback. EEG-neurofeedback is more economical and more widely available to patients than neurofeedback protocols utilising fMRI. Designs of this type may be used in the future to provide neurofeedback based on a combination of EEG and fMRI information, which may overcome some of the methodological constraints discussed earlier.

Chapter 2 of this thesis reports a multimodal study employing fMRI and resting-state ^{1}H-MRS measurements. The finding with regards to the relationship between trait anxiety, PFC Glu levels and DLPFC activation presented here is novel to the literature and provides insight into potential inefficiency in energy turnover in people with HTA. Following on from this study, is the question of how pharmacological modulation of excitatory neurotransmission may impact anxiety levels and cognition. A drug that has been employed in recent research seeking to reduce clinical anxiety levels is ketamine. Ketamine is a NMDAR antagonist, which, in small doses, has been found to reduce levels of social and generalized anxiety [277, 278]. There is also some evidence suggesting that administration of NMDAR antagonists may improve performance in attentional control tasks when Glu levels are elevated, however, it may be detrimental to cognitive performance otherwise [91, 97]. Consequently, while pharmacological studies have great potential for scientific discovery and as clinical intervention it is critical to ensure that the benefits of such an intervention outweigh possible risks.

Future work is needed that employs task-related ^{1}H-MRS measurements to capture the dynamic changes of neurotransmitter levels during task processing [85]. This would be a more suitable methodological approach to investigate whether the relationship between resting-state and task-related PFC Glu levels is influenced by trait anxiety levels. A previous study has shown that PFC Glu levels change significantly between resting-state and task-states [279] and the difference between resting-state and task-related Glu levels is altered in psychopathology [280]. However, no studies have examined how task-related changes in neurotransmitters are affected by anxiety. Thus, it is of interest if this measurement can predict anxiety levels. More research in this field is to be expected given the increased availability of ultra-high field MR scanners (7 tesla or more) in recent years, which offer greater spatial sensitivity and SNR [281].

The behavioural findings from the combined ^{1}H-MRS -fMRI study are consistent with previous literature reporting reduced *performance effectiveness* in HTA only when cognitive demands are high. This finding was not accompanied by direct differences in task-related brain activation however. Currently the body of empirical research is pointing towards task-related brain activation differing between LTA and HTA groups as a function of cognitive demands of task conditions, for example, Basten and colleagues [13] as well as Bishop [6] report increased DLPFC activation with increased task difficulty, in HTA individuals relative to LTA ones. This is possibly due to compensatory efforts due to inefficient processing. Furthermore, Bishop [6] found that in a task condition with relatively low cognitive load DLPFC activation was reduced in HTA individuals,

potentially reflecting reduced FPN functioning. However, it is unclear why this is not reflected in behavioural differences. The most plausible explanation is that their task was so easy that only minimal use of executive functions was required. Nevertheless, these findings warrant for a systematic investigation on how activation in the FPN, in particular the DLPFC, change during attentional control tasks as a function of anxiety levels and task demands on cognitive control.

With regards to the rt-fMRI-nf experiment, it is not clear, whether participants displayed reduced *performance effectiveness* at baseline, as no LTA control group was employed in this study. Furthermore, no task-related fMRI data was available, thus it is unclear if the HTA participants in this study demonstrated inefficient neural processing at baseline.

In a replication of this study it would be useful to conduct an fMRI task (e.g., Stroop task) at pre- and post-rt-fMRI-nf training to ascertain if participants demonstrated inefficient neural processing during attentional control. This would provide important information as to whether or not the effects of rt-fMRI-nf training can transfer to task-related activation during attentional control that are indicative of improvements in *processing efficiency*.

Functional connectivity-based rt-fMRI-nf is still under development as a method and as an intervention. Therefore it is of the uttermost importance that studies continue using rigid control conditions and apply relevant pre- and post- measures as suggested by Thibault and colleagues [111].

In addition, rt-fMRI-nf studies with larger sample sizes are needed to distinguish subgroups of learners and non-learners and conduct meaningful statistical comparisons between these subgroups. In doing so however, it is of great importance to establish clear criteria for categorizing participants as learners or non-learners and avoid circular inference where possible. This is not only to accurately estimate the efficacy of the intervention, but also to be able to determine factors contributing to successful self-regulation in rt-fMRI-nf training to optimise future clinical interventions.

Rt-fMRI-nf training has potential as a clinical tool for altering symptoms (e.g., [133]) or enabling patients with technology to overcome limitations they face (e.g., [282]). But the use of neurofeedback is not only relevant for clinical applications but also for cognitive enhancement and as a tool for basic research. While this research could not establish a strong connection between rt-fMRI-nf training and improved cognitive abilities, the possibility of modulating brain activation through self-regulation and observing consequential behavioural changes opens a new pathway for causal experimental research in cognitive neuroscience. Nevertheless, rt-fMRI-nf is less cost-efficient and less accessible as EEG-neurofeedback, which poses a major constraint on establishing rt-fMRI-nf interventions in clinical practice.

The findings of increased RSFC post-rt-fMRI-nf training is consistent with previous experimental work where rt-fMRI-nf training on brain activation or connectivity has resulted in wider changes in RSFC (e.g., [104, 114,

141, 162, 243]). It is yet unclear which mechanisms lead to these changes in network dynamics. Haller and colleagues [155] conducted a systematic investigation into changed in connectivity between functional networks during rt-fMRI-nf regulation as a function of the number of rt-fMRI-nf sessions participants underwent. They conducted ICA and demonstrated gradual changes in functional connectivity that are specific to the rt-fMRI-nf ROI over four training sessions. Haller and colleagues [155] speculate, consistent with other research on rt-fMRI-nf-induced connectivity changes (e.g., [283, 284]), that rt-fMRI-nf may strengthen important connections and weaken unimportant ones. These studies however do not address changes in RSFC after rt-fMRI-nf training. Investigating if changes in RSFC follow a similar pattern in relationship to the number of rt-fMRI-nf training sessions, may be a viable approach to further explore the dependencies and mechanism of dynamic brain changes post-rt-fMRI-nf.

7.5. **Conclusions**

In conclusion, this is a body of work is based on the established theoretical framework of ACT that utilises multimodal fMRI, MRS, rt-fMRI-nf, behavioural and psychometric measures to test predictions about the effects of HTA on attentional control and the associated neural substrates. Furthermore, the work reported here employs recent methodological advances in cognitive neuroscience (i.e. functional connectivity-based rt-fMRI-nf). ACT predicts impaired *performance effectiveness* and *processing efficiency* in people with HTA. This research has investigated the neural mechanisms underlying this association and in doing so has extended ACT to a neurocognitive model. In particular, the work presented

in this thesis expands on the neuroscientific understanding of impaired attentional control, specifically what is meant by impaired *processing efficiency* in people with HTA. Moreover, through the use of rt-fMRI-nf, this work has provided preliminary causal evidence for the role of dysfunctional attentional networks in HTA.

Many of the findings reported here are novel within the field of cognitive neuroscience and elaborate on the complex neural processes underlying impaired attentional control in people with HTA. However, these processes require further investigation to be fully understood. The limitations of the work are also acknowledged and a number of specific suggestions for future studies have been made.

This work has shown that the relationship between task-related DLPFC activation and resting-state PFC Glu levels may be impaired in people with HTA, possibly reflecting *inefficient processing* and reduced NMDAR functioning. Furthermore, this work has demonstrated that DLPFC-ACC functional connectivity-based rt-fMRI-nf training is a feasible intervention to modify anxiety levels by altering activation and connectivity in brain regions and networks important for attentional control processes. Much on the relationship between different substrates of neural activity and neurotransmission is yet to be understood. Similarly, more work is needed to establish the ability of functional connectivity-based rt-fMRI-nf to improve attentional control in people with HTA.

8. References

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Research Report

Altered relationship between prefrontal glutamate and activation during cognitive control in people with high trait anxiety

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ABSTRACT

Trait anxiety can affect cognitive control resulting in ineffective and/or inefficient task performance. Moreover, previous functional Magnetic Resonance Imaging (fMRI) studies have reported altered dorsolateral prefrontal cortex (DLPFC) activity in anxious cohorts, particularly when executive control is required. Recently, it has been demonstrated that cortical glutamate levels can predict both functional activation during cognitive control, and anxiety levels. In the present study we sought to investigate the relationship between trait anxiety, prefrontal glutamate levels and functional activation in DLPFC during a cognitive control task. Thirty-nine participants assigned to either low trait anxiety (LTA) or high trait anxiety (HTA) groups underwent ^{1}H-Magnetic Resonance Spectroscopy (^{1}H-MRS) to measure levels of resting glutamate in the prefrontal cortex (PFC). Participants also completed fMRI during a Stroop task comprising congruent and incongruent colour word trials. The HTA group showed reduced task performance relative to the LTA group. In the LTA group, there was a positive association between PFC Glu levels and DLPFC activation during incongruent trials. This association was absent in the HTA group. Individual differences in trait anxiety affect the relationship between PFC glutamate levels and DLPFC activation, possibly contributing to ineffective task performance when cognitive control is required.

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1. Introduction

Trait anxiety is a normally distributed personality dimension and a risk factor for anxiety and depressive disorders (Gidron et al., 2013; Sandi & Richter-Levin, 2009) characterised by intrusive thoughts, worry and difficulty in disengaging from negative material (Heeren, Bernstein, & McNally, 2018). Trait anxiety has been found to be associated with functional consequences including increased distractibility and attention problems (Bishop, 2009; Bishop et al., 2004; Eysenck et al., 2007). Indeed, the effects of trait anxiety on cognitive function have long been recognised (Berggren & Derakshan, 2013) and are accounted for by attentional control theory [ACT; (Eysenck & Derakshan, 2011; Eysenck et al., 2007)].

ACT proposes that anxiety competes for attentional resources and impairs cognitive control when executive processes are required i.e., updating, set shifting and inhibiting irrelevant or distracting information. Consequently, anxiety can impair task performance i.e., *performance effectiveness* when executive control is required. Further, ACT predicts that, even when performance effectiveness is maintained, anxiety can reduce *processing efficiency* (the quality of performance relative to use of processing or cognitive resources). In line with this prediction, functional Magnetic Resonance Imaging (fMRI) studies report increased prefrontal cortex (PFC) activation in people with high trait anxiety without concomitant improvements in performance effectiveness [i.e., processing inefficiency; (Basten, Stelzel, & Fiebach, 2011; Basten, Stelzel, & Fiebach, 2012; Fales et al., 2008)]. The PFC along with the lateral parietal cortices i.e., the fronto-parietal network (FPN), are known to be important for cognitive control (Braver et al., 2009; Sylvester et al., 2012) and support 'top-down' attention by maintaining attentional sets (Braver et al., 2009; MacDonald et al., 2000; Miller & Cohen, 2001). In particular the dorsolateral prefrontal cortex (DLPFC), comprising the middle and superior frontal gyri, has a central role in top-down cognitive control (Brosnan & Wiegand, 2017) and has been shown to have altered activation in response to tasks that require cognitive control in people with high trait anxiety [e.g., (Basten et al., 2011; Basten et al., 2012; Bishop, 2009; Fales et al., 2008)].

Despite these recent advances in our understanding of the neural mechanism involved in cognitive control, little is known about its neurochemistry and how this may be affected by individual differences in trait anxiety. Glutamate (Glu) is an excitatory neurotransmitter and its importance in cognitive control has been highlighted in animal models (Jett et al., 2017; Nardecchia et al., 2018). In humans, Anticevic and colleagues (Anticevic et al., 2012) showed that administration of ketamine, an N-methyl-D-aspartate glutamate receptor (NMDAR) antagonist, disrupts activity in FPN regions and subsequent performance during a working memory task, highlighting the role that Glu plays in cognitive control. Combining functional Magnetic Resonance Imaging (fMRI) and ^{1}H-Magnetic Resonance Spectroscopy (^{1}H-MRS), Falkenberg and colleagues (Falkenberg et al., 2012) demonstrated that the magnitude of the blood-oxygen level-dependent (BOLD) response to a task requiring cognitive control was predicted by anterior cingulate resting state Glu levels. Moreover, individual variability in resting Glu levels was related to how the brain implements cognitive control (Falkenberg et al., 2012).

These findings are important because Glu functioning is altered in some psychiatric disorders associated with cognitive control impairments (Javitt, 2004) and pharmacologically induced reductions in Glu levels have been found to alter the BOLD response during cognitive control tasks (van Wageningen et al., 2010; Yucel et al., 2007). However, whilst in vivo ^{1}H-MRS studies investigating the neurobiology of anxiety have focused on populations with diagnosed disorders [e.g., (Dager et al., 1999; Kimbrell et al., 2005; Mahmutyazicioglu et al., 2005; Whiteside et al., 2006)], ^{1}H-MRS studies in non-clinical populations in which trait anxiety is assessed as a personality dimension are relatively few in number. The first study using ^{1}H-MRS to examine metabolite levels in relation to trait anxiety reported increased PFC N-Acetyl aspartate (NAA) in participants with high trait anxiety levels but found no differences in Glu levels between high and low trait anxiety participants (Grachev & Apkarian, 2000). More recently, Modi and colleagues (Modi et al., 2014) reported that cortical Glu and combined Glu and glutamine levels (measured with ^{1}H-MRS in the anterior cingulate) were increased in participants with high relative to low trait anxiety scores and predictive of trait anxiety levels across their study cohort. Pharmacologically induced anxiety has also been reported to increase cortical Glu levels (Zwanzger et al., 2013).

Together, the studies discussed here indicate that trait anxiety can affect both DLPFC activity during cognitive control and PFC Glu levels. Whilst it has already been established that resting cortical Glu levels are important for the way the brain implements cognitive control (Anticevic et al., 2012; Falkenberg et al., 2012), to date, no studies have measured resting cortical Glu levels and DLPFC activity during a cognitive control task and examined how these are related to individual differences in trait anxiety levels. This is important because it is possible that the effects of trait anxiety on DLPFC activity (and cognitive control) are influenced by cortical Glu levels. Although the precise relationship between resting PFC Glu levels and neural activity is not fully understood, a number of studies have shown that levels of resting Glu measured with ^{1}H-MRS are related to the BOLD signal and electrophysiology measures during cognitive tasks (Allen et al., 2015; Falkenberg et al., 2012; Fusar-Poli et al., 2011; Kompus et al., 2015) and possibly mediated via NMDAR (Anticevic et al., 2012).

The aim of the present study was to investigate the relationship between trait anxiety, PFC Glu levels (using ^{1}H-MRS) and activity in DLPFC during a cognitive control task. In accordance with the predictions of ACT and findings from previous fMRI studies, we hypothesised that levels of trait anxiety would be positively associated with DLPFC activity during a cognitive control task (indicative of processing inefficiency). Based on the findings outlined above, we then tested if participants with high levels of trait anxiety had elevated levels of PFC Glu relative to a low trait anxiety group. Finally, we explored how the association between resting PFC Glu levels and DLPFC activity during cognitive control was affected by individual differences in trait anxiety levels. No part of the study procedures or analyses was pre-registered in

a time-stamped, institutional registry prior to the research being conducted.

2. Methods

We report how we determined our sample size (see Supplementary Materials). No data were excluded and inclusion/exclusion criteria are reported below. Inclusion/exclusion criteria were established prior to data analysis as were all manipulations, and all measures in the study. The raw data and materials to replicate this study or any analysis are available at Open Science Framework (DOI 10.17605/OSF.IO/PXK8Z).

2.1. Participants and assessments

Thirty-nine participants performed a colour-word Stroop task (Stroop, 1992) while functional magnetic resonance imaging and ^{1}*H-MRS* data were acquired. Participants (27 female) ranged from 18 to 37 years of age (M = 22.05 years, SD = 4.62). There were 35 right handed and four left handed participants, as assessed by the Annett Hand Preference Questionnaire (Annett, 1970). Participants were recruited from the University of Roehampton, Royal Holloway University of London and from the general public. Participants had no prior neurological or medical illness and were not using medication for anxiety or depression. The University of Roehampton Ethics Committee gave ethical approval and all participants gave written informed consent prior to taking part in the study. IQ was estimated using the Wide Range Achievement Test (WRAT-R) Reading Level 2 (Jastak et al., 1984); M = 109.15 (SD = 10.24, Range 86–131) to control for potential effects of IQ on task performance and task-related BOLD signal. Alcohol consumption and recreational cannabis use were assessed for all participants using a categorical scale (ranging from no-use to regular use). The majority of participants indicated that they used alcohol on a moderate basis and that they used cannabis never or only experimentally (see Table s1).

To assess trait anxiety, participants completed the State Trait Anxiety Inventory (STAI) (Spielberger et al., 1983). In all participants the mean score for trait anxiety was 41.33 (SD = 11.07, Range 22–78) and 33.2 (SD = 10.01, Range = 20–70) for state anxiety. This distribution of STAI trait scores is slightly higher than published norms (i.e., M = 36, SD = 10) (Spielberger et al., 1983) but comparable to scores reported by a previous study examining effects of trait anxiety on DLPFC activation (i.e., M = 43 SD = 11) (Bishop, 2009).

A median-split of STAI trait scores was used to establish low trait anxious (LTA; n = 19) and high trait anxious (HTA; n = 20) groups (see results), this dichotomization was performed to achieve greater interpretability of the results. Confirmatory analysis of behavioural and MRI data using STAI trait scores as a continuous variable are reported in the Supplementary Materials.

2.2. Experimental task

Participants performed a colour-word Stroop task adapted for MRI and used previously (Krabbendam et al., 2009). The task was programmed and presented with Microsoft Visual Basic. Participants responded with one of four fingers of their right hand to the font colour of the word presented (Red, Yellow, Blue or Green). Participants were instructed to respond as quickly and as accurately as possible while reaction times (RT) and error rates (ER) were recorded. The task consisted of a total of 100 trials, 33 congruent trials in which the font colour and meaning of the word matched, 33 incongruent trials in which the font colour and meaning of the word did not match and 34 fixation periods in which the participants saw a fixation cross. Trials were presented in a pseudo-randomized order within one functional run lasting 10 minutes. Each trial (including fixation cross trials) was presented in the middle of the screen and took 6000 msec including a period of 1300 msec before trial onset in which a blank dark grey screen was displayed. Participants then viewed a visual stimuli (i.e., congruent word, incongruent word, or fixation cross) that was presented for 700 msec. Thus participants were allowed 4700 msec from stimulus onset (700 msec during trial presentation plus 4000 msec response period) to respond i.e., responses were registered from the onset of each stimulus trial. After a response was registered the trial continued until the end of this period. No response was required in fixation cross trials.

2.3. Statistical analysis and power

IBM® SPSS Statistics Version 22 was used for the analysis of task and questionnaire data. Questionnaire and task data were considered normally distributed. A multifactorial repeated measures ANOVA with the dependent variables RT and ER in the two conditions of the Stroop task (Congruent, Incongruent) was performed. Trait anxiety group was included as a between subjects factor. A statistical significance threshold of $p < .05$ was applied throughout. To test if analyses were sufficiently powered we used G*Power (https://download.cnet.com/G-Power/3000-2054_4-10647044.html). Power calculations are reported in Supplementary Material. Furthermore, we used statistical software program JASP (JASP Team, 2016; jasp-stats.org) to compute Bayes Factor (BF_{10}) to quantify the relative likelihood of the model tested to the null hypothesis. A $BF_{10} > 3$ is considered evidence for H_1 where as a $BF_{10} < 1/3$ is considered evidence for H_0 (Jeffreys, 1939). LTA and HTA groups were compared on STAI trait and state scores, IQ estimate and age using independent samples t-tests. The groups were also compared on their alcohol consumption and cannabis use using Mann–Whitney *U* tests for ordinal data.

2.4. MRI acquisition

All MRI scans were acquired on a 3T Siemens Magnetom TIM Trio scanner using a 32-channel head coil. Structural T1 weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MP RAGE) images were acquired with a spatial resolution of 1 mm × 1 mm × 1 mm, in plane resolution of 256 × 256 × 176 slices and scanning time of approximately 5 minutes. Functional images were acquired using a full-brain, anterior-to-posterior, T2* weighted, BOLD-sensitive gradient echo planar sequence with the following parameters: TR/TE/flip angle = 2 sec/40 msec/70°, field of view 192 mm × 192 mm

and slice thickness of 5 mm giving a voxel size of 3 mm × 3 mm × 5 mm and whole brain coverage of 28 interleaved slices. Three hundred volumes were collected during the event related functional run.

2.5. ^{1}H-MRS data acquisition and analysis

^{1}H-MRS in vivo spectra were acquired from a 20 × 20 × 20 mm voxel located in the right medial PFC during rest. A voxel in the right PFC was chosen as previous fMRI studies report effects of anxiety in the right PFC (Basten et al., 2011, 2012). A medial position was chosen as lateral voxels can be harder to place due to tissue boundaries. The voxel was positioned manually by reference to an axial T1-weighted gradient echo image (Fig. 3B). Spectra were acquired using SPin ECho full Intensity-Acquired Localized spectroscopy [SPECIAL; (Mlynarik et al., 2006)] ^{1}H-MRS sequence with water suppression (TR 3000 msec, TE 8.5 msec, Phase cycle Auto, 192 averages from the right PFC voxel) in each participant (Godlewska, Near, & Cowen, 2015). Water unsuppressed spectra (16 averages) were also acquired. Six outer volume suppression slabs were applied (one on each side at 5 mm from the edge of the cubic voxel) to suppress signals originating from outside the volume of interest and to minimize motion-related image-selected in vivo spectroscopy subtraction artifacts. Spectra were analysed using LCModel 6.3-1L with the basis set consisting of 19 simulated basis spectra; alanine (Ala), ascorbate (Asc), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glycine (Gly), glutathione (GSH), glycerophosphocholine (GPC) phosphocholine (PCh), lactate (Lac), myo-inositol (mI), N-acetylaspartate (NAA), N-acetylaspartateglutamate (NAAG), phosphorylethanolamine (PE), scyllo-inositol (Scyllo) & taurine (Tau).

The basis set was simulated using FID-A (Simpson et al., 2017), for TE = 8.5 msec, magnetic field strength = 3 T and assuming ideal RF pulses. We excluded spectra with Cramer-Rao lower bounds (CRLB) > 20% as reported by LCModel. In addition to metabolite levels, line widths and signal-to-noise ratios were estimated by LCModel. All spectra had a Line Width <8 Hz and an SNR >40 (Godlewska et al., 2015).

Metabolite levels have been shown to depend on the amount of cerebral spinal fluid (CSF), gray (GMV) and white matter (WMV) within the voxel (Srinivasan et al., 2006), and inter-individual differences in cortical gray matter (Huster et al., 2007). Correlations between PFC Glutamate and GMV and WMV WMV are reported in the supplementary material. To account for these potential confounds we used the T1-weighted anatomical images to estimate the gray and white matter content of the right PFC voxel in which the ^{1}H-MRS measures were performed using GABA Analysis Toolkit (Gannet 2.0, http://gabamrs.blogspot.co.uk/) adapted to work with Siemens SPECIAL data. The segmentation was performed using "new segment" in SPM 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). CSF, GMV and WMV were then accounted for in the expression of Glu and GABA levels using LCModel (Ernst, Kreis, & Ross, 1993; Gasparovic et al., 2006); corrected metabolite levels are referred to as *Glu Corr* and *GABA Corr* using the formula $Glu\ Corr = (Glu*(43300*GMV + 35880*WMV + 55556*CSF))/(35880*(1-CSF))$ and $GABA\ Corr = (GABA*(43300*GMV + 35880*WMV + 55556*CSF))/(35880*(1-CSF))$.

Additionally, because previous studies investigating the relationship between Glu and BOLD signal during cognitive control have used metabolite ratios relative to the synchronously-acquired Cr signal (Duncan et al., 2011; Falkenberg et al., 2012) we report Glu/Cr results in a supplementary analysis (Supplementary Materials). Differences between LTA and HTA groups in right mPFC metabolite levels, as well as SNR, Line Width and CRLB were established using independent sample *t*-tests. Additionally, we calculated the BF_{10} for each comparison to assess the likelihood of the model relative to the null hypothesis. As no a-priori hypotheses for other 1H-MRS metabolite levels were stated, statistical tests for GABA, NAA, Myo-inositol, Choline and Creatine by trait anxiety group are reported in Supplementary Material.

2.6. fMRI data analysis

Functional MRI data were analysed using the Statistical Parametric Mapping software package (SPM12, Welcome Department of Cognitive Neurology, London, UK, www.fil.ion.ucls.ac.uk/spm/spm12). The anatomical and Echo Planer images (EPI) were reoriented manually based on the anterior commissure – posterior commissure axis. The images were corrected for slice timing. Motion correction was performed for functional images using six movement parameters to reduce motion artefacts. Volumes were co-registered to the high-resolution T1-weighted image and normalized into the Montreal Neurological Institute (MNI) template using parameters generated by unified segmentation of the T1-weighted structural image. The transformed data were smoothed using an 8 mm full width at half maximum (FWHM) isotropic Gaussian kernel. A high-pass filter with a cut-off of 128 sec was applied to reduce low-frequency noise.

A fixed effects general linear model (GLM) was used to model data from the Stroop task at the 1st level based on event related Congruent and Incongruent colour-word trials. The number of error trials were modelled as regressors of no interest and Fixation cross trials were modelled implicitly. The six motion correction parameters were included as regressors of no interest in 1st level models. Contrast images were created for each participant at the 1st level to examine the main effect of condition (Congruent *vs* Incongruent). The contrast Incongruent > Congruent was specified for each 1st level model to establish the effect of interference on whole brain activity at the single subject level.

These 1st level contrasts were then entered into a second-level ANCOVA to examine the main effect of task (Incongruent > Congruent trials). To assess the effect of trait anxiety on DLPFC activation we entered 1st level contrast images into a regression model in SPM v12 as power was insufficient to detect small to medium effects using an independent samples *t*-test (See Supplementary Material).

These 1st level contrasts were entered into a second-level ANCOVA with each participants trait anxiety group (LTA *vs* HTA) and PFC Glu Corr levels to examine task related activation during incongruent trials (Incongruent > Congruent), the effect of trait anxiety group on task related activation and the interaction effect for group x Glu Corr levels. Furthermore each participant's mean ER were included as a covariate of no interest to control for the effects of task performance on brain

activation as these different between LTA and HTA groups. As the effect of group on estimated IQ scores was non-significant we chose not to include estimated IQ as a covariate in ANCOVA.

Because of our a-priori hypothesis that trait anxiety would specifically be associated with increased activity in DLPFC regions during a task requiring cognitive control we used a region of interest (ROI) approach (x, y, z = +/−34, 36, 24, small volume correction (SMV) sphere = 12 mm). The DLPFC ROI was based on previous reviews of fMRI tasks that manipulate cognitive control (Duncan & Owen, 2000; Owen, 1997) and a previous study which reports a positive correlation between trait anxiety and DLPFC activity during a high load condition (Bishop, 2009). As effects of anxiety have been reported in left (Bishop, 2009), right (Basten et al., 2011, 2012; Telzer et al., 2008) and bilateral DLPFC activity (Fales et al., 2008; Karch et al., 2008) we chose to test for effects in a bilateral DLPFC ROI. Exploratory full brain analyses are reported in Supplementary Materials. For all analyses ER were included as a covariate of no interest. Significance results are reported at a threshold of $p < .05$ (FWE-peak-level). To represent results graphically parameter estimates of activation were extracted from the peak voxel in analyses. No secondary analyses were performed on the extracted values (Poldrack & Mumford, 2009; Vul et al., 2009). Plotting served the purpose of disentangling the effect revealed in the GLM.

3. Results

3.1. Trait anxiety groups

A median-split based on STAI trait scores (median = 42) was used to establish low trait anxious (LTA) and high trait anxious groups. LTA and HTA groups differed significantly on STAI trait and state anxiety scores but not in age, or estimated IQ scores. There were no significant group differences between groups in alcohol consumption or cannabis use (See Table 1).

3.2. Task performance

Error rates: Participants' ER and RT during the Stroop task are shown in Fig. 1. ANOVA revealed a significant effect of condition for ER [F (1, 37) = 24.89, $p < .001$, $\eta part^2 = .40$] with a greater ER during incongruent trials across all participants. There was also a significant effect of trait anxiety group on ER [F (1, 37) = 4.63, $p = .038$, $\eta part^2 = .11$] and significant group x task condition interaction effect [F (1, 37) = 7.59, $p = .009$, $\eta part^2 = .17$] revealing that ER were greater in the incongruent condition for the HTA group.

Reaction Times: The main effect of condition on RT was non-significant [F (1,37) = 1.84, $p = .183$, $\eta part^2 = .05$], however there was a significant effect of trait anxiety group on RT [F (1, 37) = 4.54, $p = .040$, $\eta part^2 = .11$]. Across the task the HTA group were slower than the LTA group. The group x task condition interaction was non-significant [F (1, 37) = .13, $p = .717$, $\eta part^2 < .01$]. The relative likelihood of this model compared to the null hypothesis is $BF_{10} = .29$.

3.3. fMRI: Stroop effect

Compared to Congruent trials, Incongruent trials were associated with activation in the bilateral medial superior frontal gyrus and anterior cingulate cortex, the bilateral precentral gyrus extending to the right middle frontal, and in the left middle frontal and inferior gyrus and putamen (see Fig. 2 and Table s2). There was no significant activation in the opposite contrast (Congruent > Incongruent trials) at a FWE corrected level of $p < .05$.

3.4. Effect of trait anxiety on DLPFC activity during incongruent trials

The effect of trait anxiety (STAI trait scores) on DLPFC activation was non-significant in bilateral DLPFC ROI during Incongruent > Congruent trials.

3.5. ^{1}H- MRS: Glu Corr and DLPFC activation

PFC Glu Corr metabolite levels and spectra quality control data for LTA and HTA groups are reported in Table 2. All other metabolite levels are reported in Table s3. Differences between LTA and HTA groups for right PFC Glu Corr were non-significant (relative likelihood of this model compared to the null hypothesis $BF_{10} = .64$). The correlation between Trait anxiety scores and PFC Glu Corr levels was also non-significant ($r = .25$ $p = .121$).

There was a significant interaction between PFC Glu Corr levels and trait anxiety group in the left DLPFC ROI (x, y, z = −26, 30, 18, Z = 3.60; PFWE (Peak-level) = .044) (Fig. 3C). The scatter plot in Fig. 3A shows that during incongruent trials (Incongruent > Congruent) the LTA group showed a positive association between PFC Glu Corr levels and brain activity in the left middle frontal gyrus.

In the HTA group, during incongruent trials, PFC Glu Corr levels were not associated with activation in the DLPFC ROI.

Table 1 – STAI scores, age, estimated IQ, alcohol and cannabis consumption for LTA and HTA groups.

	LTA (n = 19)	HTA (n = 20)	Analysis
STAI trait	33.05 (5.05)	49.20 (9.33)	$t(37) = -6.67$, $p < .001$
STAI state	27.79 (5.41)	38.79 (10.76)	$t(37) = -4.83$, $p < .001$
Age (years)	22.31 (5.09)	21.80 (4.25)	$t(37) = .34$, $p = .73$
Estimated IQ	109.00 (9.91)	109.30 (10.80)	$t(37) = .01$, $p = .93$
Cannabis use (Moderate)	2	0	$U = 155$, $p = .27$
Alcohol use (Regular)	3	1	$U = 183$, $p = .78$

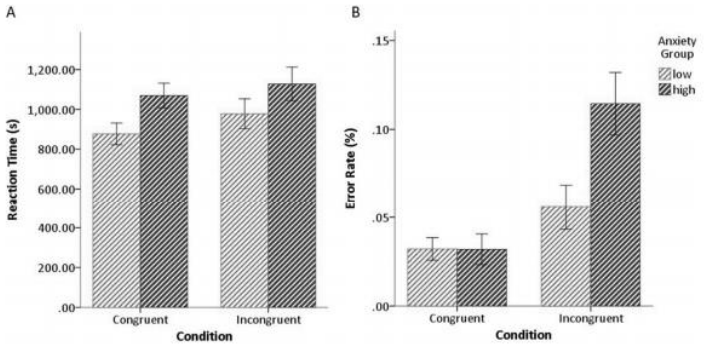

Fig. 1 – **Reaction time and error rate data for Stroop task. (A) Mean reaction time (RT) in milliseconds (msec) and (B) error rate (ER) % errors by trait anxiety group and task condition. Error bars show the standard error of the mean.**

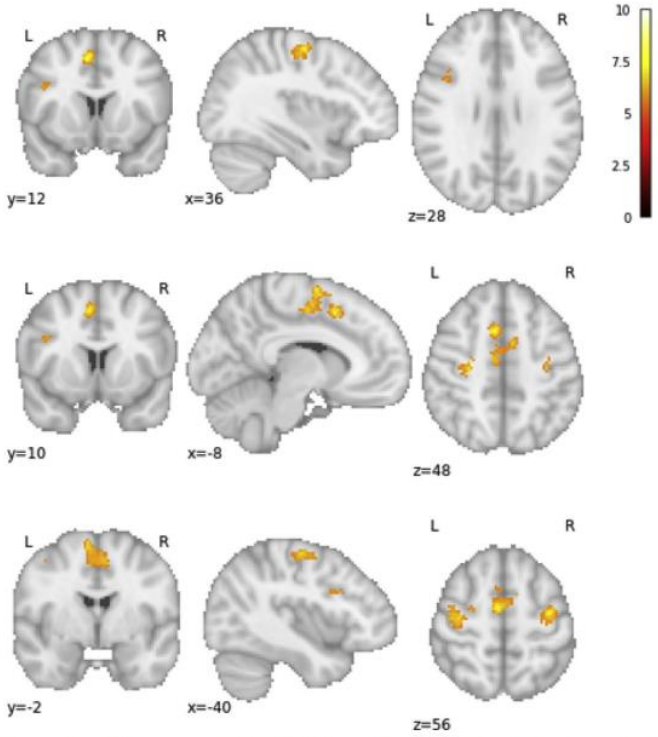

Fig. 2 – **(A) Statistical Parametric Maps in axial, coronal and sagittal sections showing the main effect of the Stroop task (incongruent > congruent) in cortical regions. Results displayed at $p < .05$ FWE peak corrected. The left side of the brain is on the left side of the image.**

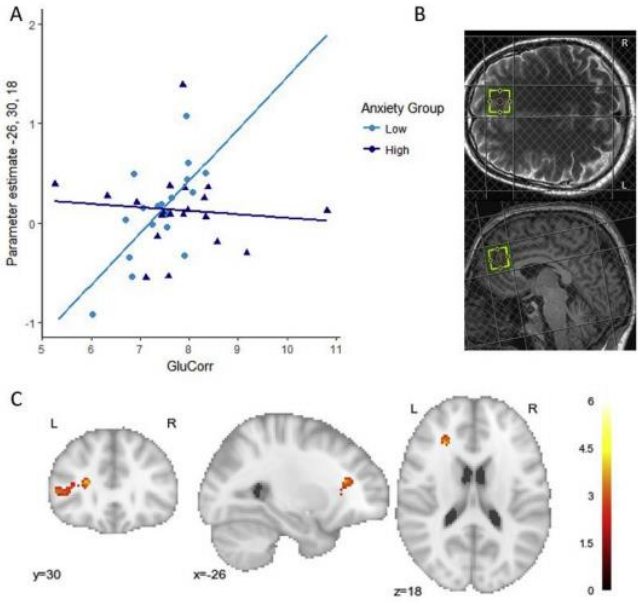

Fig. 3 – (A) Scatter plot and line of best fit showing individual contrast parameter estimates by right PFC Glu Corr levels (arb. unit) by trait anxiety group. (B) Positioning of the voxel for right medial PFC voxel for ^{1}H-MRS acquisition. (C) Statistical Parametric Map showing brain activations for trait anxiety Group x PFC Glu Corr interaction during incongruent trials at $p = .05$ FWE corrected threshold. Results displayed at $p > .005$ uncorrected for illustrative purposes.

Table 2 – Means, Standard deviations and statistical analysis/Bayes Factors for ^{1}H-MRS quality control measures, right medial PFC Glu and GABA levels (Corr &/C) by LTA and HTA groups. Metabolite levels are represented in arbitrary units.

PFC Glu/Met	LTA	HTA	Total	Analysis (LTA *vs* HTA)	
				t-test result	BF_{10}
Glu Corr	7.41 (.58)	7.80 (1.10)	7.61 (.90)	$t(37) = -1.36$, $p = .183$	.64
Glu/Cr	1.00 (.06)	1.05 (.08)	1.02 (.08)	$t(37) = -1.99$, $p = .054$, $\eta part^2 = .097$	1.44
S:N Ratio	60.00 (4.77)	60.85 (7.01)	60.44 (5.96)	$t(37) = -.44$, $p = .662$	.34
Line Width in Hz	3.53 (.79)	4.26 (1.30)	3.90 (1.13)	$t(31.67) = -2.128$, $p = .041$, $\eta part^2 = .107$	1.71
Glu CRLB	4.05 (.62)	3.85 (.67)	3.95 (.65)	$t(37) = .98$, $p = .335$	.46

This interaction effect was not accounted for by task performance (ER).

4. Discussion

The aim of this study was to examine the relationship between trait anxiety, DLPFC activation during a cognitive control task, and PFC Glu levels. Overall, participants performed the Stroop task with a high level of accuracy. As expected, during the Stroop task, error rates were greater during incongruent trials although unusually, reaction times did not differ significantly between congruent and incongruent conditions. It is unclear why this reaction time pattern was observed but it may have been due to a speed accuracy trade-off, trial/task pacing (Renaud & Blondin, 1997), or because the version of the task used in the present study used a single handed four-finger response system accounting for the relatively high reaction times observed in both congruent and incongruent task conditions. However, relative to the LTA group, the HTA group had greater ER during incongruent trials and were generally slower across the task. Reduced task performance (i.e., increased ER and RT) in the HTA group is consistent with the prediction that high levels of trait anxiety reduce *performance effectiveness* (Eysenck et al., 2007). Reduced performance effectiveness during the incongruent trial condition of the Stroop task has been reported previously in anxious individuals (Basten et al., 2011; Hopko, Hunt, & Armento, 2005) and may be related to the high cognitive control requirements of the task.

During the Stroop task, fMRI data showed that incongruent (>congruent) trials were associated with activity in the

anterior cingulate cortex (ACC) and medial superior frontal gyrus (supplementary motor area), the bilateral precentral gyrus, right middle frontal gyrus and left middle and inferior frontal gyri (as well as smaller activations in a number of subcortical regions). This finding is broadly consistent with previous fMRI studies/meta-analyses reporting functional activation during the Stroop task [e.g., (Basten et al., 2011; Derrfuss et al., 2005; Minzenberg et al., 2009; van Veen & Carter, 2005)]. It is assumed that incongruent trials increase activity in ACC, supplementary motor area, and DLPFC regions due to the increased need for cognitive control.

In people with high trait anxiety, increased DLPFC activation without improved task performance effectiveness has been interpreted as reduced *processing efficiency* (Basten et al., 2011, 2012; Fales et al., 2008). However, contrary to some previous fMRI findings, trait anxiety was not significantly associated with increased activation in the DLPFC during incongruent trials. Nevertheless, in the present study, the HTA group did demonstrate reduced performance effectiveness relative to the LTA group, suggesting that their DLPFC activation during incongruent trials may have been insufficient to perform the task effectively.

It has been reported previously that cortical Glu levels can predict anxiety levels (Modi et al., 2014) and that pharmacologically induced anxiety increases cortical Glu levels (Zwanzger et al., 2013). Examining our ^{1}H-MRS data however, there were no significant differences in PFC Glu levels between LTA and HTA groups. This may be due to our ^{1}H-MRS voxel placement, in the medial PFC, which differed from the ACC voxel placement used in these previous studies (Falkenberg et al., 2012; Javitt, 2004). We then examined how trait anxiety influenced the relationship between PFC Glu levels and DLPFC activation during cognitive control. We found a significant interaction between PFC Glu levels, trait anxiety and left DLPFC activation during incongruent task trials. This effect was driven by a positive association between PFC Glu levels and DLPFC activation in the LTA group, while PFC Glu and DLPFC activation were unrelated in HTA participants. This finding suggests a role for resting PFC Glu in DLPFC activation and is in line with previous studies by Falkenberg et al., (2012) and Duncan et al., (2011) that report resting Glu levels significantly influence how the brain implements cognitive control. Although speculative, resting PFC Glu may facilitate efficient processing during cognitive control through a higher capacity for energy turnover (Rothman et al., 2003) and/or NMDAR function (Anticevic et al., 2012) that increase DLPFC activity in line with task demands. It should be made clear however, that the relationship between resting Glu concentrations and neural energy metabolism in humans is not fully understood (Boumezbeur et al., 2010; Mangia et al., 2007). Thus, in the LTA group it is possible that such a positive relationship between excitatory neurotransmission and task related activation in the DLPFC facilitates an effective and/or efficient neural processing mechanism when cognitive control is required. On the other hand, in the HTA group, no association between resting Glu levels and DLPFC activity was observed. This could be due to effects of trait anxiety on NMDAR function. Anxiety and neuroticism (a personality construct closely linked to trait anxiety) have been shown to affect NMDAR function (Aragam et al., 2013; Garakani, Mathew, & Charney, 2006) and differences in NMDAR function can effect task-related interactions between default mode and FPN regions (Anticevic et al., 2012; Montague et al., 1994). The absence of this relationship between resting Glu levels and DLPFC activity in the HTA group may result in ineffective task performance; consistent with the predictions of ACT (Eysenck et al., 2007). Together, these findings provide new insight into how a normally distributed personality dimension such as trait anxiety can affect the relationship between excitatory neurotransmission and activation in neural regions that support cognitive control. Future work could investigate if modulation of excitatory neurotransmission can ameliorate anxiety related effects on cognition.

4.1. *Limitations*

First, we report a number of null findings which raise issues regarding the power of the study. Our power calculations (Supplementary Material) suggest that the study was sufficiently powered to detect medium to large effect sizes (.50 – .90), that have been reported previously by studies investigating the associations between DLPFC activity (Bishop, 2009), cortical glutamate levels (Modi et al., 2014) and trait anxiety. Clearly however, our study was not sufficiently powered to detect smaller effects sizes. This is important because previous studies examining the effects of trait anxiety on neurotransmitter levels for example have reported smaller effects sizes [e.g., (Grachev & Apkarian, 2000)]. Furthermore, Bayes Factors did not give a strong indication for either the null or the experimental hypothesis with regard to the relationship between trait anxiety and PFC Glu levels.

Thus, the null findings reported here need to be interpreted with some caution in as much as the study sample only provides sufficient power to detect larger effects. We cannot discount the possibility that the significant relationships between trait anxiety, DLPFC activity and/or cortical glutamate levels might be observed in a study powered to detect smaller effects sizes. Thus future studies aiming to examine the effect of trait anxiety on PFC Glu would need to recruit larger samples. It should also be noted that four of the 39 study participants were left-handed and laterality may affect stoop task performance (Christman, 2001).

Second, ^{1}H-MRS-fMRI analyses did not show any interaction effects within the right medial PFC voxel itself. Similar findings have been reported previously (Duncan et al., 2011; Falkenberg et al., 2012), where no relationship between Glu and BOLD signal was seen in the measured region. This points to a more global effect of Glu on BOLD response, exerting 'long-range' influence on other regions via glutamatergic projection (Falkenberg et al., 2012). Notably this study relies on resting state Glu measurements rather than examining changes in these metabolite levels as a result of task demands. Though the use of resting-state MRS is common practice, PFC Glu levels differ between rest and task and reflect changes in other metabolic measures and cognitive demands (Woodcock et al., 2018). Thus, future work could measure task-related differences in Glu levels to obtain a more accurate and dynamic insight into the neural basis of cognitive processes (Stanley & Raz, 2018); combined fMRI and MRS i.e., scan data

collected simultaneously is a promising method to better understand the relationship between BOLD and neurotransmitter levels in the context of task processing (Ip et al., 2017).

Third, the concept of processing efficiency/inefficiency that is central to ACT does not tell us about the precise neural mechanisms that underlie the different patterns of brain activation in people with high levels of anxiety. For example, differences in intensity and timing of neural signalling (i.e., temporal dynamics) as well as resting cerebral blood flow and metabolism would be likely to affect activation in fMRI experiments (Poldrack, 2015). However, we have shown here that excitatory neurotransmission can modulate task related activation in the PFC and that this modulation effect is perturbed in people with high trait anxiety. Finally, there is emerging evidence that cognitive deficits in people with high trait anxiety/anxiety disorders are partly due to functional network imbalances [see (Sylvester et al., 2012)]. Future work should examine how network interactions (i.e., FPN and Default Mode Network) are modulated by excitatory/inhibitory neurotransmission and how these interactions are affected by anxiety.

4.2. *Conclusions*

We have demonstrated that individual differences in trait anxiety affect the relationship between PFC Glu levels and DLPFC activation during cognitive control. This may contribute to ineffective task processing when cognitive control is required. These results need to be replicated in larger samples and more work is needed to examine how task related excitatory neurotransmission during cognitive control is affected by trait anxiety.

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CRediT authorship contribution statement

Elenor Morgenroth: Writing - review & editing, Investigation, Visualization, Formal analysis, Writing - original draft. **Natasza Orlov:** Writing - review & editing, Formal analysis. **David J. Lythgoe:** Writing - review & editing, Formal analysis. **James M. Stone:** Writing - review & editing. **Holly Barker:** Writing - review & editing, Investigation. **James Munro:** Writing - review & editing, Investigation. **Michael Eysenck:** Writing - review & editing. **Paul Allen:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition, Formal analysis, Writing - original draft.

Open practices

The study in this article earned Open Materials and Open Data badges for transparent practices. Materials and data for the study are available at https://osf.io/pxk8z/.

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Supplementary data

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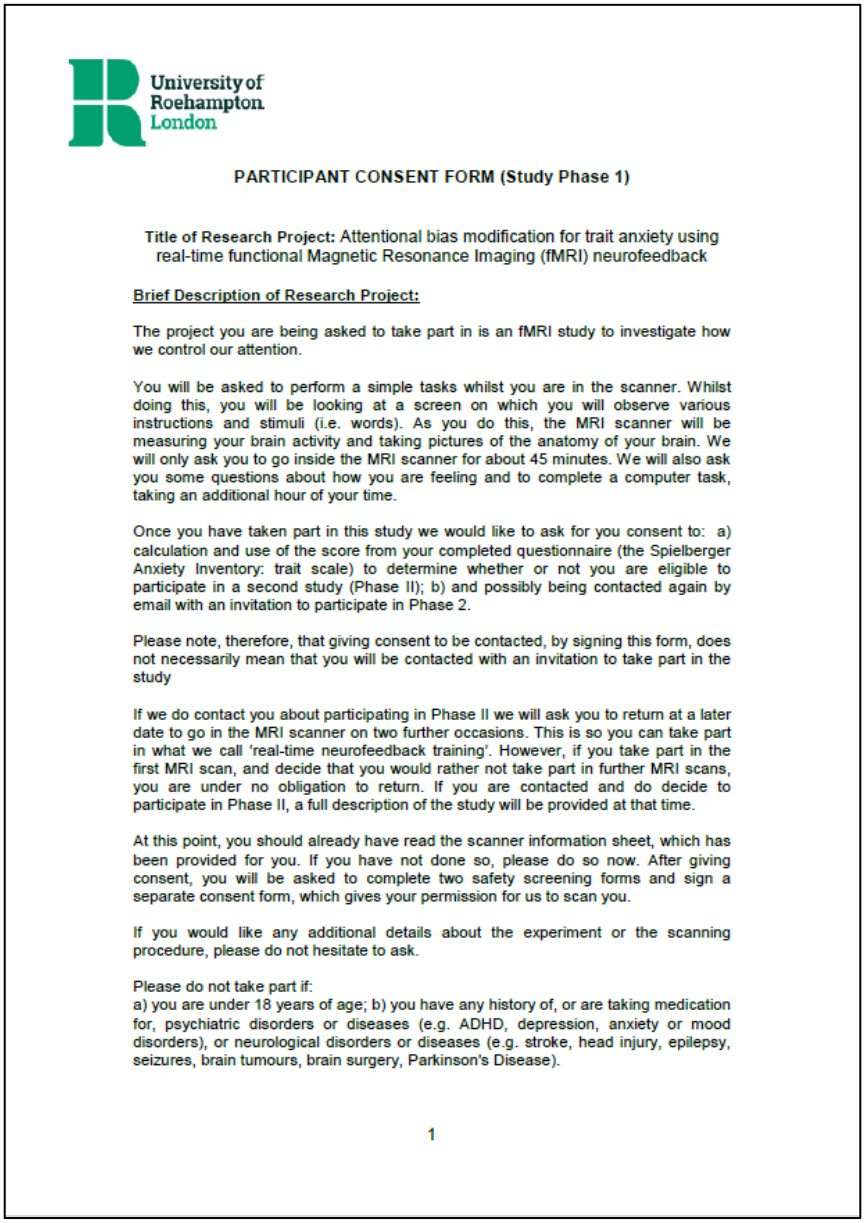
Appendix 2. Ethical Approval and Participant Consent Forms

A2.1. Ethical Approval for "Altered Relationship Between Prefrontal Glutamate and Activation during Cognitive Control in People with High Trait Anxiety"

The research for this project was submitted for ethics consideration under the reference PSYC15/182 in the Department of Psychology and was approved under the procedures of the University of Roehampton's Ethics Committee on 09/11/2015.



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PARTICIPANT CONSENT FORM (Study Phase 1)

Title of Research Project: Attentional bias modification for trait anxiety using real-time functional Magnetic Resonance Imaging (fMRI) neurofeedback

Brief Description of Research Project:

The project you are being asked to take part in is an fMRI study to investigate how we control our attention.

You will be asked to perform a simple tasks whilst you are in the scanner. Whilst doing this, you will be looking at a screen on which you will observe various instructions and stimuli (i.e. words). As you do this, the MRI scanner will be measuring your brain activity and taking pictures of the anatomy of your brain. We will only ask you to go inside the MRI scanner for about 45 minutes. We will also ask you some questions about how you are feeling and to complete a computer task, taking an additional hour of your time.

Once you have taken part in this study we would like to ask for you consent to: a) calculation and use of the score from your completed questionnaire (the Spielberger Anxiety Inventory: trait scale) to determine whether or not you are eligible to participate in a second study (Phase II); b) and possibly being contacted again by email with an invitation to participate in Phase 2.

Please note, therefore, that giving consent to be contacted, by signing this form, does not necessarily mean that you will be contacted with an invitation to take part in the study

If we do contact you about participating in Phase II we will ask you to return at a later date to go in the MRI scanner on two further occasions. This is so you can take part in what we call 'real-time neurofeedback training'. However, if you take part in the first MRI scan, and decide that you would rather not take part in further MRI scans, you are under no obligation to return. If you are contacted and do decide to participate in Phase II, a full description of the study will be provided at that time.

At this point, you should already have read the scanner information sheet, which has been provided for you. If you have not done so, please do so now. After giving consent, you will be asked to complete two safety screening forms and sign a separate consent form, which gives your permission for us to scan you.

If you would like any additional details about the experiment or the scanning procedure, please do not hesitate to ask.

Please do not take part if:
a) you are under 18 years of age; b) you have any history of, or are taking medication for, psychiatric disorders or diseases (e.g. ADHD, depression, anxiety or mood disorders), or neurological disorders or diseases (e.g. stroke, head injury, epilepsy, seizures, brain tumours, brain surgery, Parkinson's Disease).

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Right to withdraw:

There is no obligation for you to take part in the experiment, nor to finish it after you have begun to take part. You may withdraw from participation in part or all of the experiment at any point, with no explanation. You can also request for your data to be withdrawn at any point after having participated in the study. In order to do this, you may contact the investigator with your participant number (which you will find on your debrief form). It is important to note that if you decide to withdraw your data after a length of time, it may already have been published in some form.

Confidentiality

In accordance with the Data Protection Act (1998) all data relating to your participation in this study will be processed and held confidentially. Electronic forms of your data will be kept within password protected computers, whilst hard copies will be kept physically locked in filing cabinets. No one outside of the research team (detailed on this document) will have access to your data. There is one exception to this, in which possible abnormalities in the MRI image data may be sent to your GP. By signing this consent form you will be giving your consent for us to contact your GP in the unlikely event that any abnormalities are detected. No data identifiable as being yours will be published, and nothing but the raw data will contain your name.

Please tick this box if you consent to our linking your name to your data in this experiment so that it is possible to link these data to other experiments that our research group may carry out and to ask you to fill out questionnaires at a later date. ☐

Investigator Contact Details:

Professor Paul Allen
Department of Psychology
Whitelands College
University of Roehampton
Holybourne Avenue
London SW15 4JD
Paul.Allen@roehampton.ac.uk
020 8392 5147

Consent Statement:

I agree to take part in this research, and am aware that I am free to withdraw at any point without giving a reason, although if I do so I understand that my data might still be used in a collated form. I understand that the information I provide will be treated in confidence by the investigator and that my identity will be protected in the publication of any findings, and that data will be collected and processed in accordance with the Data Protection Act 1998 and with the University's Data Protection Policy.

Name .....

Signature .....

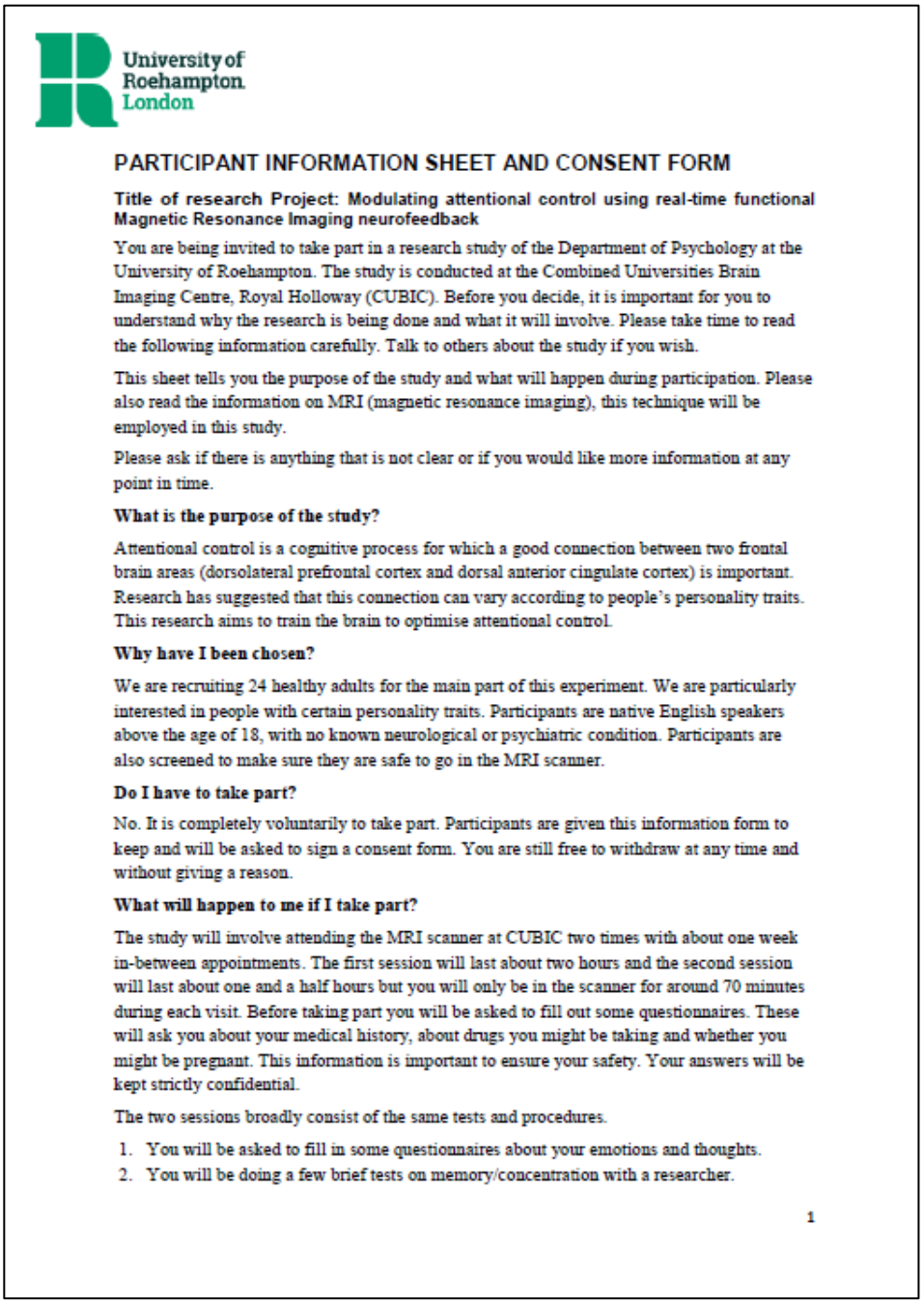
Date .....

Please note: if you have a concern about any aspect of your participation or any other queries please raise this with the investigator. However if you would like to contact an independent party please contact the Head of Department.

Head of Psychology Contact Details:
Dr Diane Bray
Department of Psychology
University of Roehampton
Whitelands College
Holybourne Avenue
London SW15 4JD
d.bray@roehampton.ac.uk
020 8392 3627

A2.2. Ethical Approval for "Modulating Attentional Control in High Trait Anxiety Using Connectivity-Based rt-fMRI-nf"

The research for this project was submitted for ethics consideration under the reference PSYC17/264 .in the Department of Psychology and was approved under the procedures of the University of Roehampton's Ethics Committee on 15/06/2017.



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PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Title of research Project: Modulating attentional control using real-time functional Magnetic Resonance Imaging neurofeedback

You are being invited to take part in a research study of the Department of Psychology at the University of Roehampton. The study is conducted at the Combined Universities Brain Imaging Centre, Royal Holloway (CUBIC). Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

This sheet tells you the purpose of the study and what will happen during participation. Please also read the information on MRI (magnetic resonance imaging), this technique will be employed in this study.

Please ask if there is anything that is not clear or if you would like more information at any point in time.

What is the purpose of the study?

Attentional control is a cognitive process for which a good connection between two frontal brain areas (dorsolateral prefrontal cortex and dorsal anterior cingulate cortex) is important. Research has suggested that this connection can vary according to people's personality traits. This research aims to train the brain to optimise attentional control.

Why have I been chosen?

We are recruiting 24 healthy adults for the main part of this experiment. We are particularly interested in people with certain personality traits. Participants are native English speakers above the age of 18, with no known neurological or psychiatric condition. Participants are also screened to make sure they are safe to go in the MRI scanner.

Do I have to take part?

No. It is completely voluntarily to take part. Participants are given this information form to keep and will be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

The study will involve attending the MRI scanner at CUBIC two times with about one week in-between appointments. The first session will last about two hours and the second session will last about one and a half hours but you will only be in the scanner for around 70 minutes during each visit. Before taking part you will be asked to fill out some questionnaires. These will ask you about your medical history, about drugs you might be taking and whether you might be pregnant. This information is important to ensure your safety. Your answers will be kept strictly confidential.

The two sessions broadly consist of the same tests and procedures.

1. You will be asked to fill in some questionnaires about your emotions and thoughts.
2. You will be doing a few brief tests on memory/concentration with a researcher.

1

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3. You will be doing a few computer based games that examine your ability to control attention some of which you will do while being in the MRI scanner.
4. A neurofeedback task in the scanner where you are asked to try to control a connection in your brain with the help of feedback. Half of the participants will form the control group that does not receive feedback based on their own brain activation. You will not be told which group you were in until you have completed the whole study. Group allocation will be decided randomly.
5. An anatomical scan and a resting state scan will be performed while you are in the MRI scanner. These scans don't require you to do anything, just to lie still in the scanner for several minutes.

We would like to call you approximately 2 weeks after the study to go through a few follow up questionnaires with you. This will take approximately 15 minutes.

Compensation to Participants

Participants will be compensated for their time at a rate of £10/hour. The experiment is expected to take 4 hours.

General Information on the MRI scan

MRI involves a strong magnetic field. Consequently, you will not be able to take part if you have a heart pacemaker, cochlear implant or other biomedical implants containing ferrous metal (including dental braces), or if you could have metal in your eye. Pregnant women cannot take part. You must not take any metal objects into the scanner or magnetic stimulation room. You will be asked about those risk factors before scanning to make sure that it is safe for you to be scanned.

The scanner environment is relatively confined in space; we therefore do not recommend you to take part if you particularly struggle with claustrophobia. You will have a chance to lay in the scanner before the experiments starts to make sure you are comfortable. There is a microphone inside of the scanner to talk to the researchers and you will also have a button that you can press to be taken out of the scanner immediately, if you feel uncomfortable. If you have any concerns before the scanning, please let us know. The total scanning time will be under 60 minutes.

It is very unlikely that we detect anything of medical importance during your brain scans, in the rare instance that we did, we would contact your GP who would then contact you. It is important to understand that if no anomaly is detected this does not mean that you have a 'clean bill of health'.

Will my taking part in the study be kept confidential?

Any information that you give us and all of the measurements that we collect will be confidential. No names will be used when the research is written up. The information will be stored in anonymous computer files and in locked filing cabinets. Names and addresses will be stored separately from other data. Only authorised researchers and staff at CUBIC will be able to link the names of participants with their data, all those people are under professional obligation to protect participant's right of confidentiality.

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We will use your data in this study and may combine your data with data that we gather in future studies. We will keep your data for 10 years and will then destroy it securely. We will comply with the terms of the Data Protection Act 1988.

What will happen to the results of the study?

The results will be written up in scientific journals and talked about at conferences. It will not normally be possible to give feedback about performance to individual participants because the data are taken away and analysed at a later date. However, the lead researcher, Elenor Morgenroth, will be happy to provide a summary of the results when they become available.

Who has reviewed the study?

This study was given a favourable ethical opinion under the procedures of the University of Roehampton Ethics Committee.

Further information

If you are concerned about safety and how the MRI procedure takes place, you can read medical websites (e.g. http://www.nhsdirect.nhs.uk/articles/article.aspx?articleId=556; http://www.ehealthmd.com/library/mri/MRI_risks.html).

If you have any further questions or concerns please ask.

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Director of Studies
Prof. Paul Allen
Department of Psychology
Whitelands College
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London; SW15 4JD
02083925147
paul.allen@roehampton.ac.uk

Please note: if you have a concern about any aspect of your participation or any other queries please raise this with the investigator. However if you would like to contact an independent party please contact the Head of Department.

If you wish to contact an independent party please refer to the Head of Department

Head of Department
Dr. Diane Bray
Department of Psychology
Whitelands College
University of Roehampton
London; SW15 4JD
02083923627
d.bray@roehampton.ac.uk

University of Roehampton London

Consent Statement:

I agree to take part in this research, and am aware that I am free to withdraw at any point without giving a reason, although if I do so I understand that my data might still be used in a collated form. I understand that the information I provide will be treated in confidence by the investigator and that my identity will be protected in the publication of any findings, and that data will be collected and processed in accordance with the Data Protection Act 1998 and with the University's Data Protection Policy.

Name .....

Signature .....

Date .....

Please tick this box if you consent to our linking your name to your data in this experiment so that it is possible to link these data to other experiments that our research group may carry out and to ask you to fill out questionnaires at a later date. ☐

4

Appendix 3. Supplementary Results to Chapter 2: "Altered Relationship between Prefrontal Glutamate and Activation during Cognitive Control in People with High Trait Anxiety"

Task Performance with Trait Anxiety as a Continuous Covariate

A repeated measures ANCOVA including STAI trait scores as a continuous covariate, revealed no significant effect of condition on ER ($F(1, 37) = 1.08$, $p = .305$), there was however a significant effect of trait anxiety on ER ($F(1, 37) = 7.01$, $p = .012$, $\eta_{part}^2 = .16$). There was also a significant trait anxiety x task interaction effect ($F(1, 37) = 5.68$, $p = .022$, $\eta_{part}^2 = .13$). The relative likelihood of this model compared to the null hypothesis is $BF_{10} = 6410.85$.

The main effect of condition on RT was not significant ($F(1, 37) = 0.12$, $p = .730$), neither was there a significant effect of trait anxiety on RT ($F(1, 37) < 0.01$, $p = .993$). There was a trend towards a significant trait anxiety x task interaction effect ($F(1, 37) = 3.16$, $p = .084$, $\eta_{part}^2 = .08$). The relative likelihood of this model compared to the null hypothesis is $BF_{10} = 0.55$.

^{1}H- MRS: Glu Corr, Trait Anxiety (Continuous Variable) and DLPFC Activation

In an additional analysis STAI trait anxiety scores were included as a continuous variable (covariate) in an otherwise identical analysis to what has been reported in the main results section. Within the DLPFC ROI there were no suprathreshold effects of trait anxiety during Incongruent > Congruent trials. There were furthermore no suprathreshold effects of Glu

Corr. There was no significant interaction between Glu Corr and trait anxiety in the left DLPFC ROI but there was a significant interaction in the right DLPFC ROI (x, y, z = 24, 32, 22, Z = 3.59; $P_{FWE\ (Peak\text{-}level)}$ = .045).

The whole brain analysis further showed a significant interaction between PFC Glu/Cr levels, trait anxiety and activity in the right anterior cingulate gyrus (x, y, z = 14, 24, 36, Z = 4.83; $P_{FWE\ (Peak\text{-}level)}$ = .034).

Trait Anxiety Group x ^{1}H-MRS Interactions Exploratory Whole Brain Analysis

The whole brain analysis revealed no regions with a significant interaction effect for PFC Glu Corr levels × trait anxiety group during incongruent trials.

^{1}H- MRS: Glu/Cr

There were no significant correlations between PFC GMV and Glu levels (r = .20 p =.21, BF_{10} = 0.41), nor between WMV and Glu levels (r = -.24 p = .14, BF_{10} = 0.57). Thus, it is unlikely that individual differences in PFC GMV and WMV influence Glu levels. There were no significant differences between LTA and HTA groups for PFC Glu/Cr. However, there was a strong trend towards higher PFC Glu/Cr levels in the HTA group relative to the LTA group (t(37) = -1.99, p = .054, η_{part}^2 = .097, BF_{10} = 1.44; Table 2).

Trait Anxiety Group x ^{1}H-MRS Interactions for Glu/ Cr

There was a trend towards an interaction between PFC Glu/Cr levels, trait anxiety group and activity in the right DLPFC ROI (x, y, z = 30, 28, 16, Z

= 3.45; $P_{FWE\ (Peak\text{-}level)}$ = .070). There was a trend towards a positive association between right PFC Glu/Cr and DLPFC brain activity in the LTA group. The whole brain analysis further showed a significant interaction between PFC Glu/Cr levels, trait anxiety group and activity in the left Inferior/Middle Frontal Gyrus (x, y, z = -48, 26, 10, Z = 4.83; $P_{FWE\ (Peak\text{-}level)}$ = .036) (Figure s1B). The scatter plot in Figure s1A shows that during incongruent trials (incongruent > congruent) the LTA group showed a positive association between right PFC Glu/Cr and brain activity in the left Inferior/Middle Frontal Gyrus. In the HTA group, during incongruent trials, PFC Glu/Cr levels were not associated with activation in this region. This interaction effect was not accounted for by task performance (ER).

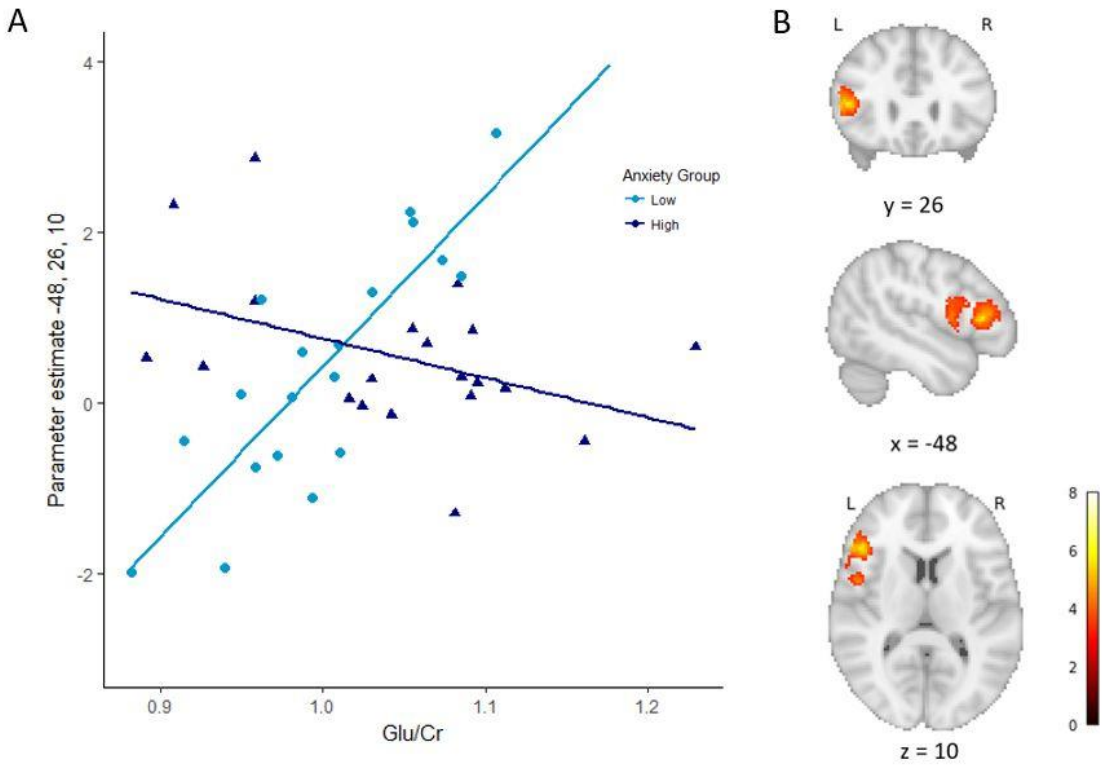

Figure s1. (A) Scatter plot and line of best fit showing individual contrast parameter estimates by PFC Glu/Cr levels (arb. unit) by trait anxiety group. Statistical Parametric Map showing brain activations for trait anxiety Group x PFC Glu/Cr interaction during incongruent trials. Results displayed at p>.001 uncorrected for illustrative purposes.

Appendix 4. Strategies Used and Self-Efficacy in the rt-fMRI-nf Training Sessions

T1			T2		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
Experimental Group					
imagining jumping up the lines/ visualisation and expecting good feedback	at first felt better not so much towards the end		tried to only think of the word over and over again/ visualize new lines coming up/ jumping from one line onto the next	first run felt improvement then not anymore/ lost focus when it stopped working	
visualise good feedback/ sing a song in head/ remember things/ lists of things	felt like had some control at some points not so much		trying to stay awake/ mentally talking loudly	didn't feel like had control	
looking at different things on the screen/ focussing on different visuals/ focus visually/ clear head of other things	felt like better control over time		same strategies as last time/ concentrating visually/ clearing mind/ close eyes briefly and open again/ imagining feedback to improve	did not feel like had control	
regulate: complicated maths/ multiplication/ change strategy/ remembering and repeating dance routines	felt like it got better up to a certain degree, at the end ran out of things to do and felt less effective	dance routines	regulate: saying colours from a song/ remembering dance routines/ maths strategies did not work/	once worked out strategies felt I got better	dance routines

T1			T2		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
			rest: turned word into "reset" and reset brain		
breathe more quickly/ think of to do lists/ cause myself stress or worry/ frowning/ think of happy things/ planning the day/ normal thinking/ stress/worry	didn't feel like control	worry	heavy breathing/ stressing myself/ thinking about stressful stuff/ worrying things	felt like improvement from last time	worry
looking at both brains/ different details on screen, closed eyes during regulate/ looked at blank spaces on screen.	felt like got better as the task went on	Looking at different parts of the brain on the screen	NA	NA	
counting/ times tables/ spelling/ recall birthdays/ mental shopping/ maths	felt under pressure a lot/didn't feel like improvement	maths	Spelling out family names/imagining remembering holidays/ spelling road names	felt like it was better than last week	spelling names
tried to remember Harry Potter plot/maths equations/ shopping list/ going through the alphabet finding a vegetable for each letter/ remember	got better towards the end	remembering animals names	remembered animal names/ shopping list with alphabet/ maths	think I got better/ probably as good as last time	alphabet shopping

T1			T2		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
animals of farm I grew up on _ faces and names					
worrying about to do list and deadlines/ stressing myself with time pressure/ things I haven't done yet/ tried singing in my head/ tried thinking of movies/ what would I do with a super power	felt like I did ok but think I could have done better/ felt more successful at the start	worrying myself with deadlines and to do things	to do lists/ singing in my head/ what would I do with superpower/ going over latin vocabulary and declination/thinking with no direction	felt like I did ok/ less stressfull than last time/ better than last time	singing and just thinking
tried stressing myself/ thinking of things I'm afraid of or dreading/ recalling angry conversations/ upsetting myself	felt like I got better over time/ felt like I did well	Upsetting myself	same as last time/ remembered argument I had/ recent argument	very hard at the beginning then started working better/ felt like I got better/ did much better in 2nd run	Remembering recent argument
thinking about swimming(participant hates swimming)/ focus only on swimming	felt like I did well and improved over time	swimming	Thinking about getting waves up/ swimming	felt worse than last time/less concentrated	
singing song in head/counting/counting in foreign language/random sums	sometimes felt like got better/felt like improved	singing	counted upwards during regulate	trial did not seem to get better	

T1			T2		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
thinking about things that happened/times tables/maths problems/singing songs/ feedback went down when didn't know song lyrics/ counting	felt like did well/maybe imroved	Times tables	thinking about past events/maths problems/to the power of series/let mind float/singing songs in my head	felt like could keep the signal up for long time	maths problems and singing
thought about break-up/ thinking of feelings about it/ thought about filming/sound reminded me of home/ thought about home/ organizing todo list	did ok/ did not feel like improvement	thinking of feelings with break up	receiting song lyrics / thinking about exgirlfriend visualizing imagining future make-belief/ thought about traffic/ remembering feeling anxious in the car/ visualizing steps of fixing car	felt like I did ok/ felt like I improved	receiting song lyrics
thinking about big future plans/ visualizing/ planning/ imagining pretending driving/ imagining drifting in car/ taking deep breaths/ seeing myself playing football	think I got better/ sometimes blanked but felt ok	imagining pretending driving	first part: imagined my team wins worldcup/ driving in car/ imagining focussing on playing football and weightlifting	felt I did well/ improved	imagining focussing on playing football and weightlifting

T1			T2		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
experimenting getting waves up and also down/ when mindwandering feedback dropped/ trying not to fall asleep/ counting/ meditative breathing focussing on breathing/ focussing on one thought pattern / thought about qualitative data analysis/ reflecting the experiment	did ok/ definitely improved over time	focussing on one thought pattern	meditative breathing caused headache so moved strategy/ sometime signal just dropped/ focussed on eyes itching/ concentrate on writing on the screen/ closed eyes while focussing/ tried not to focus on feedback too much but on strategy to get it up	not sure if I did well/ better than last time	
Control Group					
summations in head/ reciting poems/ picturing tasks from work/ picturing stroop task	didn't feel like control	poems	focus/think about fingers and toes, also tried visualising tasks at work	didn't feel like had control/ but better as last time	think about fingers and toes
rest: tried to picture black canvas/ think of nothing/ stop thoughts/ regulate: dance routines and sing along to musical theatre/ only one strategy	felt like strategy worked relatively well		regulate: listening to music and/or play movies in head fast OR music theatre songs/warmups// rest: tried to look away from screen AND/OR play	felt it got better	playing movie in head

T1			T2		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
			movies slowly/ playing movie in head / in 2nd run went back to strategy used in week1/ singing and dancing music theatre		
remembering faces/ focus visually on surroundings/ focussing on light	sometimes felt like had control	focussing on light	focus on things you could see in the scanner e.g., screws/ trying to focus on something	felt like got better as the scan went on	trying to focus on something
multiplication of big numbers/ synthesising ideas/ imagining directing/ acting/ choreography/ creating ideas/ synthesising ideas/ recollection of things did not work	found it easier at the start then harder as ran out of ideas	synthesising ideas	multiplication varying numbers/ choreographing dance sequences/ imagining the stage set/ creating/ put two things together to create something new/ did maths when ran out of things	felt like did well at the beginning but ran out of ideas and didn't feel like improvement then	creating
doing maths/ remembering anatomy/ recalling Spanish vocabulary	felt like improved but was difficult	math	mental math	felt like worked well/ improvement to session1	Mental math
counting in ones/ counting in twos/ times tables/ blinking/	felt like improvement/ got better over time	blinking	times tables/tensing and relaxing legs/ breathing rapidly/ blinking rapidly/	felt like got better/ definitely better than last time	breathing and blinking together

T1			**T2**		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
imagining waves appearing/being drawn			breathing and blinking together		
counting numbers/ thinking or sentences in another language/ focussing on breathing/ dping times 8 table during regulate/ times table	felt it got better as it went	times table	counting up in binary/ controlled breathing/ counting up in piles of two	NA	
started counting/intuitively without thinking/nothing specific/imagining more waves	felt like it worked/felt like got better		remembering holiday/ thought of my dog(dead)/ remembered situations with dog	sometimes felt like did ok/ did not feel like improvement	remembered situations with dog
counting/thinking of colours/stared at screen/ was confused for a while/ tried to relax/ zone out/ imagined on the beach focussing on waves/ pressed button	didn't feel successful		tried distressing things/imaginging fights/ imaginging being on the beach/ imagining arousing thoughts/ thought of things that made me angry	NA	

T1			T2		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
tried thinking of things that made me feel differently/ imaginging moving around/ thinking about things that mede me feel agitated/ thinking emotional memories	think I did ok	thinking emotional memories	only thought back to emotional memories/ thinking and focussing how I felt/ changed memory when stuck	felt more successful than last time	
shopping list/ imaginign and recalling groceries/ maths squared numbers/ imagined revision/ imagined emotional szenarios daydreaming/ mindpalace/ making myself emotional	felt unsuccessful	mindpalace	ignored strategies from last time/ willed waves to appear/ tried focussing hard/ tried mindpalace but didn't work	fel tbetter and more consistent than last time	willed waves to appear
imagining waves going from head to head/ counting the waves/ visualizing more waves coming/ tracing waves with eyes	felt like I did ok/ improved over time	tracing waves with eyes	traced waves from left to right and right to left/ left to right the other one didn't/ imagined waves to be there	felt ok/ felt that last time was better	tracing waves with eyes left to right
swearing in my head/ mentally frowning/screaming/making myself angry/ counting/ going through	felt like I got better	making myself angry	remembering dance routines and song lyrics/ visualizing movements and counting the beat/ dancing worked with	didn't seem to work as well as last week/ felt like had control for some of it	remembering dance routines and song lyrics

T1			T2		
Strategies used	**Self-efficacy**	**Most successful strategy**	**Strategies used**	**Self-Efficacy**	**Most successful strategy**
the alphabet/ mentally singing songs/ remember dance routines/ tried to worry/ making myself angry			chacha but not jive/ swearing/ laughing internally/ trying to command the waves saying "up" in head/ shouting encouragements at myself		
thinking about past/ visualizing planning future events/ remembering lyrics/ singing songs in my head/ trying to clear my mind together with singing songs	did ok/ got better some time	trying to clear my mind together with singing songs	visualizing/ imagining past events worked best/ tried feeling different emotions/ singing songs in head	felt like better than last time did good	imagining past events worked best

Appendix 5. Custom Python Scripts Developed for "Modulating Attentional Control in High Trait Anxiety Using Connectivity-Based rt-fMRI-nf"

A5.1. Custom Python Script to Calculate Baseline and Maximum Connectivity Values from Functional Localizer Data

```
# -*- coding: utf-8 -*-
"""
@author: Elenor Morgenroth
support: Janis Stolzenwald
This script is to calculate ConnectivityBaseline and ConnectivitMax from
Functional Localizer
"""

"""
Function partial_corr()
Date: Nov 2014
Author: Fabian Pedregosa-Izquierdo, f@bianp.net
Testing: Valentina Borghesani, valentinaborghesani@gmail.com

Partial Correlation in Python (clone of Matlab's partialcorr)

This uses the linear regression approach to compute the partial
correlation (might be slow for a huge number of variables). The
algorithm is detailed here:

    http://en.wikipedia.org/wiki/Partial_correlation#Using_linear_regression

Taking X and Y two variables of interest and Z the matrix with all the variable
minus {X, Y}, the algorithm can be summarized as

    1) perform a normal linear least-squares regression with X as the target
    and Z as the predictor
    2) calculate the residuals in Step #1
    3) perform a normal linear least-squares regression with Y as the target
    and Z as the predictor
    4) calculate the residuals in Step #3
    5) calculate the correlation coefficient between the residuals from
    Steps #2 and #4;

    The result is the partial correlation between X and Y while controlling
    for the effect of Z
"""

import numpy as np
from scipy import stats, linalg
import pandas as pd
import matplotlib.pyplot as plt


def partial_corr(C):
    """
    Returns the sample linear partial correlation coefficients between pairs
    of variables in C, controlling for the remaining variables in C.

    Parameters
    ----------
    C : array-like, shape (n, p)
    Array with the different variables. Each column of C is taken as a variable

    Returns
    -------
    P : array-like, shape (p, p)
    P[i, j] contains the partial correlation of C[:, i] and C[:, j] controlling
    for the remaining variables in C.
    """
```

```
    C = np.asarray(C)
    p = C.shape[1]
    P_corr = np.zeros((p, p), dtype=np.float)
    for i in range(p):
        P_corr[i, i] = 1
        for j in range(i + 1, p):
            idx = np.ones(p, dtype=np.bool)
            idx[i] = False
            idx[j] = False
            beta_i = linalg.lstsq(C[:, idx], C[:, j])[0]
            beta_j = linalg.lstsq(C[:, idx], C[:, i])[0]

            res_j = C[:, j] - C[:, idx].dot(beta_i)
            res_i = C[:, i] - C[:, idx].dot(beta_j)

            corr = stats.pearsonr(res_i, res_j)[0]
            P_corr[i, j] = corr
            P_corr[j, i] = corr

    return P_corr

def reject_outliers(data):
    m = 2
    u = np.mean(data)
    s = np.std(data)
    filtered = [e for e in data if (u - m * s < e < u + m * s)]
    return filtered

def partial_corr_xy(C):
    return partial_corr(C)[0,1] # return pCorr xy,z

def partial_corr_withWindow(data, size): # where size is the window size
    rows = data.shape[0]
    out = np.zeros(rows)
    for i in range(0,rows - size):
# move the window over the data table and save respective partial corr to "out"
        data_window = data[range(i, i+size)]
        out[i] = partial_corr_xy(data_window)
    return out


# lets load some data (replace this with your actual data)
data_input = pd.read_csv('C:/CUBIC/data/001HH/001HH_LocalizerConnectivity.csv')
data_gen = data_input.copy().groupby('Condition')
wind = 20

x = np.zeros(1)
y = np.zeros(1)
z = np.zeros(1)

var = ['DLPFC','ACC','noise',001]

for key, value in data_gen:
    if key == 'Condition':
        x = np.asarray(value[var[0]])  # lets load columns by their title
        y = np.asarray(value[var[1]])
        z = np.asarray(value[var[2]])
        data = np.vstack((x,y,z)).transpose() # combine collumns to matrix
pcorr = partial_corr_withWindow(data,wind)
```

2

```
#Option to save series of correlation values
#with open('E:\PhD_Localizer\pcorr.txt','wb') as f:
#    np.savetxt(f,
#               np.c_[pcorr],
#               delimiter =',',
#               newline ='\n',
#               header = 'Partial correlation between DLPFC,
#                         dACC and White Matter',
#               fmt='%f'
#               )
#f.close()

pos = pcorr[(pcorr > 0)]

#Some plotting options
#a = np.hstack(pos)
#plt.hist(a, bins='auto')  # plt.hist passes it's arguments to np.histogram
#plt.title("Positive values")
#plt.xlim(0,1)
#plt.show()

posq=reject_outliers(pos)
#a = np.hstack(posq)
#plt.hist(a, bins='auto')  # plt.hist passes it's arguments to np.histogram
#plt.title("Positive values outliers removed")
#plt.xlim(0,1)
#plt.show()
#print
#print 'Percentiles for all positive values'
#for l in [0,10,20,30,40,50,60,70,80,90,100]:
#    pospercents =np.percentile(pos,l)
#    print pospercents
#print
print 'Percentiles for positive values after outliers (+/- 2sd) removed'
for l in [0,10,20,30,40,50,60,70,80,90,100]:
    pospercents =np.percentile(posq,l)
    print pospercents

#print
#print 'Percentiles for original data'
#for l in [0,10,20,30,40,50,60,70,80,90,100]:
#    percentiles = np.percentile(pcorr,l)
#    print percentiles
```

A5.2. Custom Python Script for Real-Time Calculation of Feedback in Conjunction with TBV

```
"""
@author: Ellie Morgenroth
adapted from MONTF_NFB_short.py (FLorian Krause)

This script is for one neurofeedback run
"""

import os
import time
from expyriment import control, stimuli, design, io
from expyriment.io.extras import TbvNetworkInterface
from expyriment.design.extras import StimulationProtocol

# SETTINGS
HOST = 'HPLP174'
exp = 'ELLIE_NFB'
wd = 'Z:\Neurofeedback'

os.chdir(wd)

PORT = 55555
TR = 1000 # TR in ms
PREP_SCANS = 0
SKIP = 10
CONDITION_LENGTH = 45
REST_LENGTH = 25
NR_REPETITIONS = 6

SHOW_REST_FEEDBACK = False
START = 0  # In percent!
THERMOMETER_SEGMENTS = 10

# INSTRUCTIONS
instructions_header = "You are about to start the Feedback Run {0}"
instructions_content = \
"""
Take note of the instructions at the bottom of the screen.
During REGULATE blocks the number of waves reflects your brain connectivity.
Try to achieve as many waves as possible.
"""
end_header = "The Feedback Run {0} is now finished"
end_content = \
"""
"""

# DESIGN
exp = design.Experiment("Feedback")
exp.add_data_variable_names(["Run", "Condition", "FeedbackTrial",
                             "TriggerTime", "StimulusTime", "MeanSignal"])
control.initialize(exp)

trigger = exp.mouse
###
tbv = TbvNetworkInterface(HOST, PORT, timeout=TR/2)

protocol = StimulationProtocol("volume")
protocol.add_condition("rest")
protocol.add_condition("task")
feedback_displays = [stimuli.Picture(os.path.join(wd,'Stimulus\Visual_0.jpg')),
                     stimuli.Picture(os.path.join(wd,'Stimulus\Visual_1.jpg')),
```

1

```
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_2.jpg')),
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_3.jpg')),
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_4.jpg')),
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_5.jpg')),
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_6.jpg')),
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_7.jpg')),
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_8.jpg')),
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_9.jpg')),
                    stimuli.Picture(os.path.join(wd,'Stimulus\Visual_10.jpg'))
                    ]

for stim in feedback_displays:
    stim.preload()

rest_displays = [stimuli.Picture(os.path.join(wd,'Stimulus\Visual_Rest.jpg'))]

for stim in rest_displays:
    stim.preload()

# RUN
control.start()
i = io.TextInput('Baseline: ')
if i == 999:
    i = 0
baseline = float(i.get())
i = io.TextInput("Max: ")
if i == 999:
   i = 1
_max = float(i.get())

i = io.TextInput("Run: ")
run = float(i.get())

volume = 1
for repetition in range(NR_REPETITIONS):
    for condition in ("rest", "task"):
        block = design.Block()
        block.set_factor("Condition", condition)
        if condition == "task":
            block.set_factor('feedback', '1')
            protocol.add_event(condition,
                               volume,
                               volume+CONDITION_LENGTH - 1,
                               1
                               ) #prt file
            volume += CONDITION_LENGTH
            for length in range(CONDITION_LENGTH):
                trial = design.Trial()
                block.add_trial(trial)
        elif condition == "rest":
            block.set_factor("Rest", "-1")
            protocol.add_event(condition,
                               volume,
                               volume+REST_LENGTH - 1)
            volume += REST_LENGTH
            for length in range(REST_LENGTH):
                trial = design.Trial()
                block.add_trial(trial)

        exp.add_block(block)
```

2

```
baselines = []
old = [None, None]
current_trigger = 0
stimuli.TextScreen(instructions_header.format(run),
                   instructions_content).present()
#eeg_marker.send(0)
exp.keyboard.wait()
stimuli.TextLine("Waiting for first MR trigger...").present()
for prep_scan in range(PREP_SCANS):
    trigger.wait_press()
trigger_time = exp.clock.time
for block in exp.blocks:
    for trial in block.trials:
        if exp.clock.time - trigger_time < TR:
            trigger.wait_press()
            trigger_time = exp.clock.time

        else:
            trigger_time += TR
        current_trigger += 1
        if trial.id == 0:
            if block.get_factor("Condition") == "rest":
                rest_displays[0].present()
            elif block.get_factor("Condition") == "task":
                block.get_factor("Condition")
                exp.keyboard.check()
        if block.id == 0:
            mean = -1
        time_point = None
        while True:
            exp.keyboard.check()
            time_point, rt = tbv.get_current_time_point()
            if time_point >= current_trigger - 1:
                break
        if current_trigger >= 2:
            while True:
                if current_trigger < 20:
                    break
                exp.keyboard.check()

                try:
                    mean,rt2 = tbv.get_partial_correlation_at_time_point(20,
                                                            current_trigger-2)
                    print mean
                    mean = mean[0]
                    break
                except:
                    pass
            if mean is not None:
                displays = None
                if block.get_factor("Condition") == "rest":
                    if block.id > 0:
                        displays = rest_displays
                        displays[0].present()
                elif block.get_factor("Condition") == "task":
                    displays = feedback_displays
                    feedback = int(round(
                            ((mean-baseline) / (_max-baseline)) * 10.0))
```

3

```
                    if feedback > 10:
                        feedback = 10
                    elif feedback < 0:
                        feedback = 0
                    print feedback
                    displays[feedback].present()

            exp.data.add([run,
                          block.get_factor("Condition"),
                          trial.id,
                          trigger_time,
                          exp.clock.time,
                          mean
                          ])


stimuli.TextScreen(end_header.format(run), end_content).present()
exp.keyboard.wait()

if not os.path.exists("protocols"):
    os.makedirs("protocols")

timestamp = time.strftime("%Y%m%d%H%M", exp.clock.init_localtime)

protocol.export2brainvoyager(
        "{}_Sub{0}_Feedback_{1}_param_{2}".format(
                                                  exp,
                                                  repr(exp.subject).zfill(2),
                                                  run,
                                                  timestamp
                                                  ),
         "protocols/MOTNF_S{0}_Feedback_{1}_param_{2}".format(
                                                 repr(exp.subject).zfill(2),
                                                 run,
                                                 timestamp
                                                 )
                                )

exp.data.rename(
        "{}_Sub{0}_Feedback_{1}_{2}.xpd".format(exp,
                                                repr(exp.subject).zfill(2),
                                                run,
                                                timestamp
                                                )
                )
exp.events.rename(
        "{}_Sub{0}_Feedback_{1}_{2}.xpe".format(exp,
                                                repr(exp.subject).zfill(2),
                                                run,
                                                timestamp
                                                )
                )

control.end()
```

4